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UNIVERSITY OF
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ASSESSMENT OF ARTERIAL STRUCTURE AND FUNCTION IN SOUTH ASIAN ISCHAEMIC STROKE SURVIVORS IN THE UNITED KINGDOM

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Copyright statement

Originality statement & Contributions

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person. I was directly involved in recruiting all the patients to the Stroke study and performed all the vascular measurements by myself, after obtaining required practical training. An interpreter and a research nurse helped me in recruiting South Asians to the study. I performed all the laboratory assays under direct supervision of Sandwell Research Unit & ASCOT centre laboratory staff, who trained me to perform ELISA assays independently. I was involved in performing all the relevant basic statistical tests with my supervisors' guidance and the university statistician helped me with performing advance statistical analysis.

I forward this thesis to

My beloved parents

especially my father, the late Mr H.M. Gunarathne

for his valuable inspiration, wisdom and morals who always reminded me the great Sinhalese proverb

"Ugatha mana shilpayami mathu rakena"

(Education is the only asset which never can be stolen but always inspire your life)

the meaning of which I do appreciate

"My Utmost"

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Glossary

Apo AI	Apolipoprotein AI
Apo B	Apolipoprotein B
AF	Atrial Fibrillation
ANOVA	Analysis Of VAriance
ACEI	Angiotensin Converting Enzyme Inhibitor
AUC	Area Under Curve
BMI	Body Mass Index
CABG	Coronary Artery Bypass Graft
cGMP	Cyclic Gunaylate Mono Phosphate
CI	Confidence Interval
CIMT	Carotid Intimal Medial Thickness
CHD	Coronary Heart Disease
CRP	C-Reactive Protein
CT	Computed Tomography
CV	Coefficent of Variation
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
EC	European Caucasian
ECG	Electrocardiograph
ELISA	Enzyme Linked Immuno Sorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
ESC	European Society of Cardiology
FH	Family History
FMD	Flow Mediated Dilatation
FPG	Fasting Plasma Glucose
GTN	Glyceryl Tri Nitrate
HDL	High Density Lipoprotein
HR	Hazard Ratio
HO	Haem-Oxygenase 1
hS-CRP	High Sensitivity C-Reactive Protein (assay)
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IHD	Ischaemic Heart disease
IL6	Interleukin 6

LACI	LACunar Infarct
LDL	Low Density Lipoprotein
Lp(a)	Lipoprotein(a)
LPL	Lipoprotein Lipase
MAP	Mean Arterial Pressure
MI	Myocardial Infarction
MRS	Modified Rankin Score
MRA	Magnetic Resonance Angiogram
MRI	Magnetic Resonance Imaging
NIDDM	Non Insulin Dependent Diabetes Mellitus
NO	Nitric Oxide
NO_3	Nitrate
OR	Odds Ratio
OxLDL	Oxidised Low Density Lipoprotein
PVD	Peripheral Vascular Disease
PACI	Partial Anterior Circulation Infarct
POCI	POsterior Circulation Infarct
PWV	Pulse Wave Velocity
RI	Reflective Index
ROC	Receiver Operating Characteristics
SA	South Asians
SD	Standard Deviation
SE	Standard Error
SMR	Standardised Mortality Ratio
SI	Stiffness Index
SNSS	Scandinavian Neurological Stroke Scale
TACI	Total Anterior Circulation Infarct
TC	Total Cholesterol
TNFα	Tumour Necrosis Factor alpha
UK	United Kingdom
VLDL	Very Low Density Lipoprotein
vWF	Von Willebrand Factor
WHO	World Health Organization
WHR	Waist Hip Ratio

Publications that emanated from the thesis

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Introduction to the thesis

This thesis addresses several questions concerning the underlying pathophysiology (arterial structure and function) of ischaemic stroke amongst the migrant South Asian (originating from the Indian subcontinent) populations living in the United Kingdom, through the use of clinical and epidemiological approaches that included: cross sectional, retrospective, prospective, case control and laboratory-based studies. **Chapter one** explores the epidemiology of cerebrovascular disease amongst South Asians, examining in particular, disease prevalence, incidence, mortality rates, distribution of cardiovascular risk and possible pathophysiological explanations for ethnic variations in disease rates. **Chapter two** briefly outlines the study hypotheses and relevant study designs. All the epidemiological, pathophysiological and laboratory-based methodologies used in the process of data collection are systematically discussed in this chapter including the methods used in statistical analysis. The **third chapter** examines the distribution of conventional cardiovascular risk profile and stroke prevalence amongst South Asian stroke survivors over the last decade and specifically examines the magnitude of impact of these risk factors on stroke mortality in South Asians. As a first step towards understanding the pathophysiology of cerebrovascular disease amongst this higher risk population, **chapter four** centres upon a discussion of the structural properties of the vessel wall in the healthy South Asian population compared to that of European Caucasians. A comprehensive analysis of arterial stiffness and endothelial function in blood vessels amongst South Asian stroke survivors is presented in **chapter five** and enumerate the reasons for aberrant vessel wall characteristics amongst South Asian stroke survivors, specifically examining endothelial nitric oxide axis parameters and influence of metabolic, inflammatory and oxidative stress related indices on the vascular remodelling process and its mediation and subsequent determination of higher arterial stiffness and impaired endothelial function. The **final chapter** summarises the conclusions of each study and discusses potential areas of future research in cerebrovascular disease amongst South Asians.

Abstract

Appraisal of current literature reveals an increased burden of cerebrovascular disease amongst the migrant South Asian population living in the United Kingdom (UK) and a paucity of data concerning stroke pathophysiology among members of this ethnic group. To address these issues, a number of cross-sectional, retrospective, and prospective case control studies were performed, examining the stroke characteristics and its underlying vascular pathophysiology. The Digital Volume Pulse (DVP) analysis technique and laboratory-based studies were used to examine the structural and functional vessel wall abnormalities.

In the initial registry-based studies, looking at a large stroke population, prevalence of diabetes was more common in South Asians. Ischaemic stroke mortality was associated with diabetes, which raises the question of how diabetes accelerates stroke in this population. In cross-sectional studies, we were able to test hypotheses related to structural and functional vessel wall characteristics, where South Asians demonstrated to have higher arterial stiffness and impaired endothelial function. Glycaemic status was independently associated with both impaired endothelial function and increased arterial stiffness amongst South Asian stroke survivors. Analysis of the endothelially-derived nitric oxide production cascade amongst South Asian stroke survivors, demonstrated a significantly lower endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) levels compared to European Caucasians. A separate sub study of this thesis validates the use of DVP analysis technique within the work of this thesis and its clinical utility for finer cardiovascular risk stratification.

In conclusion, the epidemiological and pathophysiological findings of this thesis have important implications for UK South Asians and their high cerebrovascular disease burden. Observations within this thesis may aid targeted approaches for the alteration of cardiovascular risk profiles, which may aid the prevention of strokes in this high-risk community. The findings of this thesis would require validation in a large population-based community studies.

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Chapter 1 : Background

1.1 What is "Ethnicity"?

The word "ethnicity" stems from the Greek word "ethnos" meaning a race or nation (Just, 1989) and is conventionally defined as "a group of people having common racial, cultural, religious, or linguistic characteristics especially designating a racial or other group within a larger system" (The Oxford English Dictionary). Previously, many scholars have attempted to define ethnicity using, as a basis for their definition, its social, anthropological and subjective contexts. According to Webber et al. (1922) both the subjective and collective elements within the concept of ethnicity are, for example, explained as properties of "people who entertain a subjective belief in their common descent because of similarities of physical type or of customs or both, or because of memories of colonization and migration". According to Barth (1969), the concept of ethnicity was perpetually renegotiated between the minority group and the majority population by both external ascription (labelling by the majority population) and internal self-identification.

According to the above definitions, the terms "ethnic" and "ethnicity" can be referred to minority groups whose members belong or are perceived to belong, as a result of distinct shared characteristics, which include cultural traditions, languages, diet, life styles, geographical and ancestral origins when compared to the majority. However, ethnic identity is not continuous, permanent, universal or inherent to individuals. Instead, ethnic identity stems from and depends upon interactions between individuals. Such interactions give rise to common beliefs, values, norms and practices.

Ethnicity is associated with the geographical location of the majority of the group, placing the emphasis more upon place than the characteristics of members of the

group (Webber, et al., 1922). According to Weber et al. (1922), even if members in different ethnic groups migrate to various geographical locations, as long as a balance is maintained and the numbers are kept virtually constant or increasing, an ethnic group will continue to exist despite its new location.

Ethnicity is an important variable in epidemiology and health research (Ahdieh and Hahn, 1996). In analyzing differences in disease patterns within and between populations, certain diseases have a greater incidence in some ethnic groups when compared to others (Bhopal, et al., 1999). The reasons appear to be multi-factorial. Therefore, a greater understanding, through research, of those relationships which are posited to exist between ethnicity and disease will provide greater understanding of disease aetiology and enable interventions to be based upon greater knowledge than may currently be the case. Although ethnicity and race are often thought of as being controversial variables (Aspinall,2002; Senior and Bhopal, et al., 1994) in epidemiology and research, ethnicity (however this is defined) is a recognized risk factor in many cardiovascular and cerebrovascular pathologies including stroke (Chaturvedi, 2001).

1.2 Who are South Asians?

The people whose ancestry is in the countries of the Indian subcontinent, including India, Pakistan, Nepal, Bangladesh and Sri Lanka are encompassed by the term "South Asian" (Bhopal, et al.,2004). Even though some countries, such as Afghanistan, Iran, Myanmar and Tibet, are geographically contiguous with this region, their inhabitants are not considered to be South Asian due to social, cultural, religious and historical perspectives (see below) (Bhopal, et al.,1991).

1.2.1 Social, cultural and economical perspectives of native South Asians

Seventy percent of the South Asian population live in rural areas and most rely on agriculture for their livelihood (The World Bank, 2007). According to the Global Hunger Index, South Asia has a higher rate of child malnutrition when compared to other countries (The Global Hunger Index, 2006). The people in this region have, as a common factor, placed women in a lower status. These factors collectively may account for the lower socio-economic status which is seen equally across all the South Asian countries.

The differences in composition of races and religions in each country may suggest that each country is different from the other. For example, 80% of the population of India and Nepal are followers of Hinduism whilst the majority in Sri Lanka and in Bhutan are Buddhists. Languages also echo this heterogeneity within the region. Even though a large number of languages are spoken in the region, most of the languages show similarities in words and grammar syntaxes because of the common origins of the languages.

Even though these differences prevent the South Asians from being uniform and homogeneous, common features in the main religions, and resulting influences in lifestyles have outweighed the force towards divergence. Due to imperfections in the free flow of information and free will of the common citizen, national and regional identities still exist. As such, more weight can be placed on the argument that a South Asian identity exists where the inhabitants of South Asia share common factors that influence their lives. Therefore, even if people in South Asia migrate from the region and people from outside South Asia move in to the region, the ethnic identity “South Asian” will remain where such a South Asian ethnic group or the minority group is identified in relation to the world population as a whole (Oppenheimer, 2001). We can identify that

when South Asian people migrate to any other country, they may adopt behaviors and practices of the majority but the effects of their previous behaviors will ensure that they remain distinct.

1.2.2 Dietary habits and food intake in native South Asian populations

Diet has an important impact on cardiovascular disease. Traditional diets subscribed to by people of Indian origin are largely low in fat and high in carbohydrate (Achaya, 1994). Whilst religion and languages share the same roots and have common features despite the seeming variation, diet too shares common features in this region. South Asian food is called “Desi Cuisine” which indicates an Indian pedigree since most of the foods in Asian countries are derivatives of Indian food even though each food item may have, to a certain extent, its own national or regional identity (Achaya, 1994). For example, food items made from wheat flour dough are common throughout the countries of South Asia even though some additional ingredients, such as scraped coconut or ghee or different spices and condiments such as sesame seed, may be added in different regions to suit their palettes (Appadurai, 1988). This diet is popular among rural populations but may promote glucose intolerance and features of the metabolic syndrome. Another notable commonality of South Asian diet is the decreased consumption of beef and/or pork for religious reasons. Pork is prohibited by Islam, whilst the cow is held to be sacred by followers of Hinduism. Taking the life of an animal is discouraged in Buddhism. Since most of the populations in each country are Hindus, Moslems or Buddhists, consumption of meat is low in this region (Banerji, 2006). South Asian dietary habits have also been influenced by the colonial history and European food habits are also commonly observed in this region. On the other hand, diets associated with urbanisation and migration in Indians is thought to include a higher fat proportion, which may promote CHD. Both diets are thought to promote adverse effects through dyslipidaemias.

Chadha et al., (1995) conducted a dietary based survey on urban families living in South Asia (Delhi and on rural families from Haryana). Average carbohydrate intake was significantly higher in the rural group compared with the urban population. Total dietary fat intake was not significantly different between the rural and urban cohorts. However, fat intake was lowest in the low socio-economic group and highest in the middle socio-economic group. Consumption of saturated fatty acids was highest in the rural group as was the intake of monounsaturated fatty acid.

1.3 South Asians in the UK

Migration of people from the Indian subcontinent followed two periods of mass migration to numerous destinations leading to a wide global dispersion of South Asian migrants (Jain, 1993). The post war economic recovery in Western countries (UK) generated a great demand for labour. Unlike the indentured labour system, Britain allowed the migration of whole families and communities from India during the 1960s and 70s through the British Nationality Act of 1948 (Clarke, C., 1990). This enabled Indians and other members of the Commonwealth to come to Britain, secure employment and gain citizenship. Prior to this, academia had been the only route through which Indians migrated to the UK.

Estimates from the 1991 census showed that Indians formed the largest ethnic minority group, representing 1.7% of the ethnic composition of England and Wales followed by Black Caribbeans at 1% ((Kohli, 1988, National Statistics UK, 1994). The UK arrival and social features of migrants originating from the Indian subcontinent are shown in Table 1.2. Indian migrants cluster in urban areas and reside predominantly in London, the West Midlands, West Yorkshire and Lancashire.

According to the latest statistics, Four percent of the total UK population comprised of South Asians (National Statistics UK, 2004). South Asians form the largest

ethnic minority group (20%) in inner city areas such as our local Sandwell and Birmingham area (National Statistics UK, 2004).

1.4 Epidemiology of ischaemic stroke in South Asians

1.4.1 Introduction

Stroke is a continuing yet preventable cause of significant morbidity and mortality in the Western world (Olesen and Leonardi, 2003) and will rapidly reach epidemic proportions in modernising countries such as those across Asia (Manton, et al., 1988; Tolonen, et al., 2002). Of particular concern is that mortality from ischaemic stroke in the United Kingdom (UK) is at least 1.5 times more common amongst those of South Asian origin (Wild and McKeigue, 1997) when compared to the general population. One striking observation amongst the global diaspora of South Asians is that stroke is not common except for migrants living in the UK (Tolonen, et al., 2002).

Why is this increased stroke mortality evident amongst South Asian subjects in the UK? Cultural and socio-economic factors may account for part of this excess. Rates of cardiovascular disease (CVD) in Fiji (Collins, et al., 1996), Singapore (Hughes, et al.,1990) and South Africa (Seedat, et al.,1990) are highest amongst migrant Indians, but are largely explained by the excess of coronary heart disease (CHD). In the UK, CVD amongst South Asian migrants is due to both heart disease and stroke, suggesting that an environmental basis may, in part, underlie the increased risk of stroke in this population.

Thus, an examination of those modifiable risk factors that appear particularly prevalent amongst ethnic minority groups (South Asians in particular) may explain how a rapid transition from subsistence living to a ‘Westernised lifestyle’ may provide the exposure needed for the activation of inherent ethnic traits culminating in ischaemic stroke. In addition, rates of stroke in both urban and rural India are lower than those seen in metropolitan cities (Dhamija and Dhamija, 1998). This excess of stroke mortality

amongst South Asians in Britain is aligned with diabetes mellitus and hypertension, both of which show unique patterns of individual susceptibility and severity in this population (Patel, et al., 2006). Whilst there have been two comprehensive reviews on stroke epidemiology in 1992 (Bonita, et al., 1992) and 2003 (Feigin, et al.,2003) highlighting both geographical and ethnic variations in stroke mortality and incidence and case fatality rates, neither of these reviews include studies on South Asians. Hence, there is pressing need for a comprehensive review of stroke epidemiology in the South Asian population, members of which are known to carry an increased burden of stroke, and inequitable health therein (Gill et al.,2007).

This chapter deals with the specific epidemiological review of ischaemic stroke in people originating from the Indian subcontinent ('South Asians'), with particular attention to the increased risk that is exclusively manifest amongst migrants settled in the United Kingdom.

1.4.2 Literature search methodology

The aim of this chapter is to present a comprehensive and systematic overview of the available literature on cerebrovascular disease in migrant South Asians and selected studies from their counterparts in South Asian countries. The term "migrant South Asian" was used to represent people originating from the Indian subcontinent (India, Sri Lanka, Pakistan, Nepal and Bangladesh). In keeping with the above definition on South Asians, a MEDLINE and EMBASE search using the terms (MeSH): (South Asians) OR (Indians, Sri Lankans, Bangladeshi, Pakistani, Nepali) AND (Stroke) OR (Cerebrovascular accidents) was performed. Due to the paucity of published articles in peer-reviewed journals between the period selected initially (1990-2005), the literature search was extended to include articles published between the years 1940-2005,

including selected published abstracts from South Asian countries, studies with different study designs and variable stroke rates.

In total, 31 representative articles were selected, based upon 28 cross sectional and three prospective studies describing at least one demographic, aetiological or psychosocial aspect of stroke epidemiology. No formal statistical analysis (such as meta-analyses) was employed, and data were analysed descriptively, because of the observed methodological heterogeneity across the selected studies.

1.4.3 Incidence of ischaemic stroke in South Asians

The calculation of stroke incidence among South Asian populations is hampered by the lack of population-based studies. Data concerning the incidence of stroke in this population group is based mainly upon three studies conducted by Bhattacharya et al. (2005), Rao et al. (1971) and by Hsu et al. (1999) (Table 1.3).

The first reported study in India compared stroke incidence between urban and rural populations over a two year period (1969-1971). In this study by Rao et al. (1971), the crude incidence rate amongst the urban population was 19.4/100000 compared to 13/100000 of the rural population. Age adjusted incidence rates and age specific incidence rates were not available for comparison. A prospective study by Bhattacharya et al (2005) provided other evidence on stroke incidence amongst the rural Indian population. In this study (n=20717), 128 first ever stroke cases were detected over a five year period. The average annual crude incidence rate was 123.57/100,000 persons. After age adjustment to the 1990 general population, the adjusted rate was 262/100000. In comparison the age-adjusted incidence of stroke in this study was much higher than that of Western studies (Brown, et al., 1996) conducted during the same time period such as those from the USA(145/100000), Italy(136/1000000) and USSR(226/100000) (Feigin, et al., 2003). In contrast to Western studies, age-standardized stroke incidence rates were

marginally higher in the female population. In this study by Bhattacharya et al. (2005) mean age of stroke onset was 61 years. In common with Western studies, age specific incidence rates showed a progressive increase in incidence, especially in the 5^{th} to 7^{th} decades (374 to 782/100000).

One study which compared stroke incidence rates amongst South Asians living at moderate altitudes with that of individuals living at lower altitude in India showed lower incidence rates amongst those living at a higher altitude (Mahajan, et al., 2004). The reason for this apparent discrepancy is not clear. Moreover this study was based on hospital admission rates and cannot be compared with the rates of true population based incidence studies. Differences in altitudes are also likely to be apparent in migrants living in different countries across the globe, but it is unclear whether this could explain differences in stroke rates between migrant and non-migrant South Asians.

As mentioned, there is only one reported population based stroke study in the migrant South Asians (n=74) in the United Kingdom. This study, by Hsu et al. (1999) was based on a survey of 23 general practices with patients (n=129225) in Leicestershire during 1996. In this prospective study, calculated crude incidence rates were higher in Caucasians (low Asian practices) compared to South Asians (high Asian practices) (283.2 vs. 111.18 per 100,000 persons). However, reported age specific incidence rates were comparable. The indirect sampling method used in this study does not allow any direct comparison of the true incidence rates and may have promoted an underestimation of the reported incidence rates amongst South Asians.

None of the mentioned studies fulfil the criteria for ideal incidence studies (Feigin, et al., 2003). Paucity of data, different study designs and the lack of description of case ascertainment methods do not allow direct comparison between these studies. More importantly, none of these studies clearly present crude and age specific rates in the

published manuscripts, which make detailed comparisons and interpretation of the data very difficult. Other factors such as aging of population in South Asian countries and increased reporting may also confound observed increase in overall stroke incidence seen with time in these countries.

Within the limitations of the afore-mentioned studies, the incidence rates of stroke have exponentially increased from 13 per 100,000 persons (in 1969) to 123 per 100,000 (in 1993) in South Asians, against a decline in rates for other Western populations. According to the WHO Global Burden of Diseases Study, the annual stroke incidence is projected to increase by 1.5% in 2015 (Essati, et al., 2004) particularly amongst the Asian populations in developing countries. Hence, there is an urgent need to identify those factors and environmental exposures that have accelerated stroke risk among South Asians in the UK.

1.4.4 Prevalence of ischaemic stroke in South Asians

Eleven prevalence studies are included (Table 1.4). The majority of these studies have been carried out in different areas of India from 1968 to 2001. However, there are no stroke prevalence data available from other South Asian countries except Pakistan (Asian Acute stroke Advisory Panel, 2000). Two studies directly compared the prevalence of stroke amongst migrant South Asians when compared to other ethnic groups (Venketasubramanian, et al., 2005; Anand, et al., 2000) .

The crude prevalence rates of stroke among South Asians living in India vary from 52 to 842 per 100,000 for all ages (Table 1.4). As expected, age specific prevalence rates also show an exponential rise with age in all studies. In common with stroke studies within Western countries, the majority of the studies (except the study reported by Banergee et al (2001), demonstrated higher prevalence rates in males when compared to females. prevalence rates have increased three to five fold over the last four decades

(for example, 56 per 100,000 as reported by Abram et al (1971) in 1968, when compared to 334 per 100,000 which was reported by Banerjee et al (2001). More importantly, rates of stroke prevalence have been demonstrated to have significant heterogeneity across studies carried out in different parts of India. Distances span some 2400km between the East and West of India, similar to that across the North and South. The reasons for heterogeneity across studies carried out in different parts of India could be multi factorial.

Table 1.1: Studies comparing stroke incidence rates amongst South Asians

Author	Year	Method of diagnosis of stroke	Study methodology / Case ascertainment	Sample population	Investigated epidemiological aspect	Key findings
Rao et al.	1971	CT diagnosis and clinical diagnosis based on WHO definition.	Unclear	India, Madras (n= 258576)	Comparison of stroke incidence rates between urban vs. rural population.	Crude incidence rate:13 per 100000.Age adjusted rates are not available. Males had higher rates(15.2) compared to Females(10.8) and Urban rates(19.4) were higher than Rural rates.(13)
Bhattacharya et al.	2005	CT diagnosis and clinical diagnosis based on WHO definition	Prospective door-to-door survey	India, West Bengal (n=20842)	Stroke incidence rates in India	Age-adjusted rate : 262 per 100000. Females had higher rates (274) compared to males (253). Age specific rates showed a proportional increase with age
Hsu et al.	1999	CT diagnosis and clinical diagnosis based on WHO definition	Prospective general practitioner registry based case monitoring	UK, Leicestershire (n =129225)	Comparison of stroke incidence rates between SA vs. EC	Age adjusted stroke incidence rates were similar between two ethnic groups. Crude rates (283.2 vs. 111.18) were higher in EC, age specific rates showed a proportional increase with age
Mahajan et al.	2004	CT diagnosis	Prospective study based on consecutive hospital admissions	India,Himalaya (High altitude) (n= not mentioned)	Stroke incidence rates at high altitudes	Males had higher rates compared to Females and rates were higher than low altitudes. Incidence rates are based on hospital admission rates. Therefore not comparable with other studies.

SA=South Asian, EC=European Caucasian, M=Male, F=Female, CT=Computer Tomography, UK=United Kingdom, WHO=World Health Organization.

Within India, there is a mixture of persons with Indo-Aryan, Dravidian and Mongolian origins and their lifestyles vary with the religious and climatic conditions of the area they inhabit. For example, there are more than 400 languages used across India of which 20 of them are considered as 'official' languages. These different areas experience varying degrees of socio-economic influence on physical and social morbidity, which underlie practical difficulties in the epidemiological analyses of diseases across the Indian subcontinent. For example, the study by Bharucha et al (1988) reported a prevalence of 842 per 100,000 compared to the 147 per 100,000 reported by Banergee et al(2001) using a similar study design during the same period. In addition, the prevalence of stroke in urban India appears to be higher (Razdan, et al., 1989) than that seen in rural areas such as Haryana where the prevalence rate was only 92 per 100,000. (Saha, et al., 1993). In Pakistan, Jafar et al (2006) demonstrated comparatively higher prevalence rates compared to other Asian studies. However, the targeted sampling approach used in this study may have contributed to the disproportionate prevalence rates reported in the literature.

There are only two studies that have directly compared the prevalence rates of stroke in South Asians compared to that of other Asians and Europeans. The study by Venketasubramanium et al (2005) compared the prevalence of stroke amongst migrant South Asians when compared to Chinese and Malays, and found that crude as well as age adjusted prevalence rates were similar amongst all ethnic groups (SA:362, Malay:332 and Chines:376). The response rate of this study was only 67% and it remains unclear whether this apparent similarity between the ethnic groups depicts epidemiological reality. A Canadian study by Anand et al (2000) demonstrated lower prevalence rates amongst South Asians, whereas Caucasians had the highest stroke prevalence rate (1800/100000) compared to South Asians (300/100000) and Chinese (600/100000).

Table 1.2: Studies comparing stroke prevalence rates amongst South Asians (I)

Author	Year	Method of diagnosis of stroke	Study methodology / Case ascertainment	Sample population	Investigated epidemiological aspect	Key data findings
Anand et al.	2000	Self reported history or clinical diagnosis by physician	population based, cross sectional	Canada (n=985)	Comparison of stroke prevalence rates between SA vs. EC and Chinese	Crude prevalence rates were similar amongst ethnic groups: SA: 300, EC: 1800 and Chinese: 600 per 100000 population (P=0.13).Age adjusted rates are not reported.
Venketa-Subramaniu m et al.	2005	Clinical diagnosis using WHO definition	Population based, cross sectional	Singapore (n=15606)	Comparison of stroke prevalence rates between SA vs. Malaysian and Chinese	Crude as well as age-standardized rates were similar amongst ethnic groups (SA:362, Malasian:332,Chinese:376) per 100000 population
AASAP	2000	Unclear	Based on national health records of individual country	Nine Asian countries (India was the only SA country)	Comparison of stroke prevalence between nine Asian countries	Crude prevalence in India ranges from 90-222 per 100000. Thailand and Taiwan had higher reported prevalence rates (690 and 1430) per 100000
Health Survey England	2005	Clinical diagnosis using WHO definition	Population based door to door health survey	Stratified proportionate sample from general population	Comparison of stroke prevalence rates amongst ethnic groups	Crude prevalence in South Asians (Indian: 1100,Pakistani:1800, Bangladeshi:1800) were lower than EC(2400) per 100000 population

SA=South Asian, EC=European Caucasian, M=Male, F=Female, CT=Computer Tomography, UK=United Kingdom, WHO=World Health Organization.

Table 1.3: Studies comparing stroke prevalence rates amongst South Asians (II)

Author	Year	Method of diagnosis of stroke	Study methodology / Case ascertainment	Sample population	Investigated main epidemiological aspect	Key data findings
Bharucha et al.	1988	Clinical diagnosis by a neurologist	Population based door to door survey	India Bombay (n=14,010)	Stroke prevalence	Crude prevalence was 842 per 100000 population. Age specific rates were higher in men
Banergee et al.	2001	Clinical diagnosis by a neurologist or CT imaging	Population based cluster survey	India Calcutta (n=50291)	Stroke prevalence	Crude prevalence was 147 and age adjusted rate was 334 per 100000 population. Females had higher prevalence in all age groups
Razdan et al.	1989	Clinical diagnosis by a neurologist	Population based survey	India, rural Kashmir (n=63645)	Stroke prevalence in rural India	Crude prevalence was 143 per 100000. 11% of the people were in the 15-40 age group.
Saha. et al.	1993	Clinical diagnosis using WHO definition	Community based house to house survey	India, Eastern (n=20842)	Stroke prevalence in rural India	Crude prevalence was 147 per 100000 and age specific prevalence showed an increasing frequency with age.
Dhamija et al.	1998	Unclear	Population based survey	India, Haryana (n=51165)	Stroke prevalence in rural India	Crude prevalence was 92 per 100000, age specific rates and population characteristics not available
Jafar et al.	2006	Self reported history	Community survey and target sampling	Pakistan (n=500)	Pakistan Karachi	Crude prevalence was 4800 per 100000, age specific rates and population characteristics not available
Abraham et al.	1971	Unclear	Population based survey	India, Madras	Prevalence	Crude prevalence was 56 per 100000, age specific rates and population characteristics not available

SA=South Asian, EC=European Caucasian, M=Male, F=Female, CT=Computer Tomography, UK=United Kingdom, WHO=World Health Organization

Similar findings have been reported from a recent health survey in the UK. The prevalence rates of ischaemic stroke in migrant South Asians were significantly lower (Indian: 1100, Pakistani: 1800 Bangladeshi: 1800 and European Caucasians: 2400 per 100000 population) compared to the general population (Department of Health, 2005). These results appear to contradict the higher CHD prevalence which has been observed in South Asian populations (Gupta, et al., 2008). In addition, rates reported in the Indian studies also appear to be lower than the reported rates in other Asian (200-769/100000) (Liu, et al., 2007) as well as Western studies (400-1000/100000). Although the reasons for this disparity may well be multifactorial, part of the explanation rests with higher mortality rates and the consequent under-estimation of prevalence rates in the South Asian population. However, poor study designs, small sample sizes, poor case ascertainment methods, recording and publication biases and lack of quality in data presentation in the published studies may further restrict critical appraisal, interpretation and comparison of stroke rates between countries as well as amongst ethnic groups.

1.4.5 Stroke mortality in South Asians

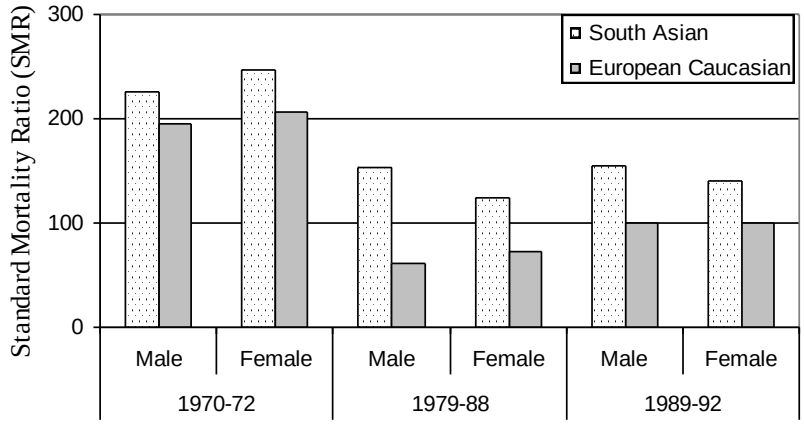
Ischaemic strokes accounts for more than 10% of all deaths globally and are the third most common cause of mortality in developed countries (Bonita, et al., 1992). There are ethnic variations reported in stroke mortality, mainly comparing Caucasians and Afro-Caribbeans living in developed countries (Kissela, et al., 2004). A small number of cross sectional studies from UK and official statistical audits from India provide data on rates of ischaemic stroke mortality in the South Asian population (Table 1.6), (Figure 1.1).

Table 1.4: Studies comparing stroke mortality rates amongst South Asians

Author	Year	Source of data of stroke	Study methodology / Case ascertainment	Sample population	Investigated main epidemiological aspect	Key data findings
Bhopal et al.	2005	National mortality data by country of birth 1994-1997	population survey and registry analysis	UK Newcastle	SMR, comparing EC vs. SA	SMR was higher in SA (155) compared to EC (100) and Bangladeshis had highest SMR(281)
Wild et al.	1997	National mortality data by country of birth 1970-1992	Cross sectional survey and registry analysis	UK	Trends in SMR comparing EC vs. SA, AfroCar.	AfroCar males had highest SMR (271) followed by SA (226) in 1970-72. Rates have significantly dropped in all ethnic groups in 1989-92 period.
Balarajan et al.	1991	National mortality data by country of birth 1970-89	Cross sectional survey and registry analysis	UK	Trends in SMR comparing EC vs. SA	SMR was 53% higher in SA males compared to EC in 1970. SA males showed the lowest reduction in stroke rates (3%) compared to EC (28%) between 1972-83 period
Balarajan et al.	1997	National mortality data by country of birth 1988-92	Cross sectional survey and registry analysis	UK	SMR comparing SA Bangladeshi men vs. EC	Bangladeshi men had highest SMR (267) compared to EC
Hsu et al.	1999	Prospective study	Registry based case monitoring	UK, Leicestershire	30 day and 1 year stroke mortality	SA had a better survival at 30days (78%) as well as after one year (68%) follow up compared to EC (68% and 55%)

UK=United Kingdom, SA=South Asian, EC=European Caucasian, AfroCar=Afro-Caribbean, M=Male, F=Female, SMR=Standard Mortality Ratio

Figure 1.1: Secular trends in the Standard Mortality Ratio amongst South Asians in the UK 1970-1992



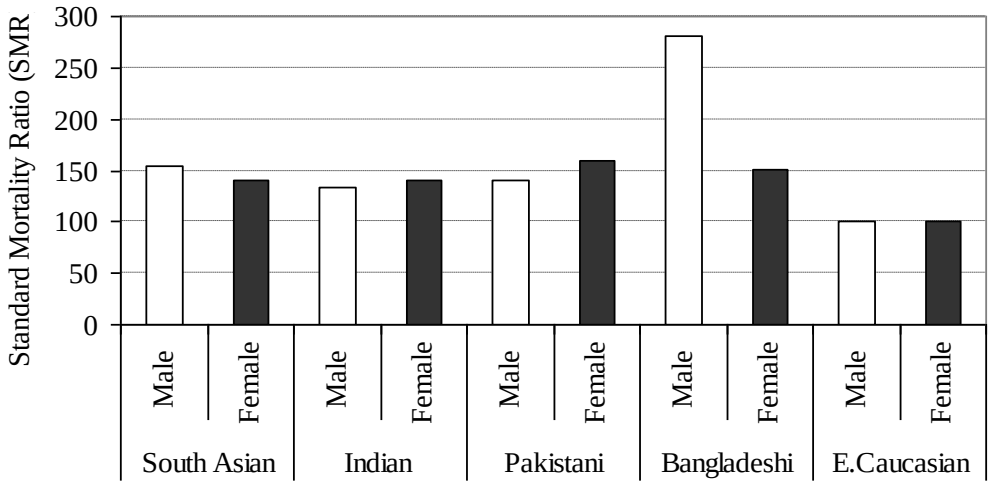
* Based on national census data: Wild, et al (1997); Bhopal, et al (2005); Balarajan, et al.(1997); Gill, et al (2007).

Official census data from the UK in 1981, 1991 and 2001 have persistently shown inequalities in ischaemic stroke mortality amongst the South Asian population. According to the three cross sectional studies (Bhopal, et al., 2005; Balarajan, et al., 1991) based on the national census data in the UK, the average Standard Mortality Ratio (SMR) in South Asians were 55% and 41% higher in males and females respectively when compared to the Caucasian population (Figure 1.1). The average SMR for the South Asians between the age groups 20-69 years was 155 for males and 141 females. While rates of mortality from stroke have been declining in the UK population as a whole between 1970-1992, (Figure 1.1) the rate of decline was ten times less in South Asians compared to the Caucasian population.

Gill et al (2007) reported an intra-ethnic variation in ischaemic stroke mortality in South Asians (Figure: 1.2, below). It was noted in particular that Bangladeshi born men living in the UK had the highest mortality rate (SMR: 201) compared to other South Asian populations (SMR: 155). There were no significant gender differences reported in the stroke mortality rate amongst the South Asian population except amongst the

Bangladeshi population, where males had a significantly higher SMR compared to females.

Figure 1.2: SMR amongst South Asian subgroups in the UK: 2004



Based on national census data: Based on national census data: Wild, et al (1997); Bhopal, et al (2005); Balarajan, et al (1997); Gill, et al (2007).

Based on a one-year ischaemic stroke mortality study by Hsu et al. (1999) ischaemic stroke mortality was significantly lower in South Asians compared to the Caucasian population. There are limited studies which report ischaemic stroke mortality rates in the Indian subcontinent. Short-term (30-day) stroke mortality in a rural hospital-based study in India was 32% in 1968, which declined to 12% in 1982 (Dalal, et al., 2006). The Global Burden of Disease study reported that 61,900 deaths (6.5% of all deaths) were accounted for by strokes in the year 1990. In the UK based South Asian stroke study (Hsu, et al., 1999) only 78% South Asians survived at the end of 28 days. In comparison, reported mortality rates from western countries (Geramny 11%, Norway 10%, France 12% and Australia 12%) are significantly lower than that of South Asian stroke populations (Feigin, et al., 2003). Possible explanations for the observed differences amongst South Asians may include variations in the management, type and severity of ischaemic stoke compared to Caucasian stoke populations.

However there is no significant evidence to support the assertion that stroke mortality rates in the migrant South Asian population differs when compared to non-migrant populations in their country of origin, mainly due lack of comparison studies between these two groups. However, higher rates of stroke mortality in migrant South Asians in the UK are consistent with reported higher risk of CHD in these subjects.

1.4.6 Predictors of Stroke Severity in South Asians

Ethnicity has been reported as an independent predictor of stroke severity (Jones, et al., 2000). Evidence from USA-based studies reveals a direct relationship between stroke severity and other contributory factors such as in-hospital mortality, duration of hospital stay, poor Glasgow Coma Scale (GCS) score and incontinence during acute presentation (Kuhlemeier and Stiens, 1994). Apart from the Leicestershire Stroke Study (Hsu, et al., 1999), which reported a significant association between development of incontinence during the acute stage and 28 day stroke mortality in South Asians, there appears to be no other studies available to infer a relationship between the severity of stroke and South Asian ethnicity.

1.4.7 Stroke sub-types in South Asians

Population-based studies have shown significant differences in stroke sub-types in the White populations compared to that of the Black populations (Friday, et al.,1989; Wityk, et al.,1996; Gorelick, et al.,1984). The majority of published South Asian stroke studies have not enumerated the stroke classification or type of infarction, perhaps due to limited imaging facilities in South Asian countries. This may also be explained by the limited number of population-based studies.

Of the few available studies (Table 1.5), one Pakistan-based stroke study showed prevalence rates of 66% for ischaemic strokes, 21% for intra-cerebral haemorrhage and 8.3% for subarachnoid haemorrhage (Syed, et al.,2003). One study carried out in India

also showed a significant increase in the prevalence of hemorrhagic strokes of 32% (INTERSTROKE-India Study Group, 2006). Two other studies from South Asian countries also showed similar distribution of stroke sub-types (Vohra, et al., 2000). In the absence of direct comparative studies, prevalence rates from the above three studies imply a higher risk of hemorrhagic stroke in the South Asian population compared to that of the Caucasian population (21% (Ebrahim, et al., 2006) vs. 6.4% (Bamford, et al., 1990). The only two South Asian studies which classified their stroke population according to the TOAST taxonomy found a higher prevalence of lacunar strokes (42.7% and 68%) compared to large vessel infarctions (26% and 10%) (Syed, et al., 2003; Deleu, et al., 2006) and lower occurrence of cardio-embolic strokes (6.1%). Whilst lower prevalence of cardio-embolic strokes may be directly related to significantly lower atrial fibrillation rates amongst South Asians (see section below). The higher occurrence of lacunar infarctions in the South Asian population has not been previously highlighted, but the increased prevalence of diabetes and hypertension in the South Asian population probably accounts for this excess risk of developing small vessel disease (Kaul, et al., 2002). Further studies are therefore necessary amongst South Asian stroke survivors and the wider South Asian population.

1.4.8 Management of stroke amongst South Asians

Variations in standards of stroke management are held to vary depending upon the ethnicity of the sufferer (Goldstein, et al., 2003). As with other ethnicity-related epidemiological data concerning stoke, most of the evidence comes from literature comparing Black, Hispanic and White populations in the USA.

In the majority of reported studies, ethnic minority groups appeared to experience a lower standard of care when compared to the indigenous white population (Stansbury, et al., 2005). Nonetheless, there are inconsistencies in all stages of the data at different

levels of stroke management (from acute presentation, imaging, pharmacological treatment and rehabilitation to patient discharge). Indeed Bhopal (2005) suggests that, the standards of care for stroke survivors in the UK during all phases of the illness are lamentably poor whatever one's ethnicity (Bhopal and Rahemtulia, 2005).

There are only a small number of studies that examine issues of stroke management in the migrant South Asian population in the UK (Bourke, et al., 2006). There have been very few studies available for analysis from any South Asian countries (Brainin, et al.,2007). It is held that there are delays in access to health care which affect the South Asian population and result in South Asians being less likely to receive appropriate treatment (Brainin, et al., 2007). This is despite Hsu et al (1999) reporting that South Asians were more likely seek early hospital treatment following an acute stroke compared to Caucasians. Similarly, according to two Indian based studies only 29.5% (Nandigam, et al., 2003) and 14.5% (Pandian, et al., 2004) reached hospital within three hours (cut off time for thrombolysis) following an ischaemic stroke. However these rates are no different to that of Western populations (Bourke, et al., 2006). One of the contributory factors compounding poor standards of stroke care particularly amongst South Asians is the existence of barriers to communication (Feder, et al.,2002) which can delay treatment as a result of an inability express symptoms or communicate effectively, which results in a consequent inability to establish a medical history of the event. Feder et al. (2002) also described the influence of the socio-economic status of South Asians and its influence on the provision of culturally appropriate and sensitive health care. In common with the ethnic minority groups in the USA, South Asian stroke patients in the UK are more likely to have radiological investigations than the Caucasian population (Nandigam, et al., 2003). For example, a South Asian stroke study carried out among members of the Bangladeshi population showed a higher number of both CT and MRI scans being performed in this group compared to that of Caucasians (Nandigam, et al.,

2003). Possible explanations include atypical presentation on admission and the unreliability of clinical symptoms resulting in the deferment of a correct clinical diagnosis until further investigations are carried out. There was, however, no disparity observed in the pattern of referrals for rehabilitation or carotid endarterectomies (Nandigam, et al., 2003).

The evidence from the USA (Bourke, et al., 2006) as well as the UK (Nandigam, et al., 2003) suggests that the management of stroke among ethnic minority groups including South Asians was hampered by lack of adequate service provision. This situation is made worse because of non-individualized treatment strategies and relatively small number of trials involving South Asians, particularly in cardiovascular research (Gammon and Gunarathne, 2008). This relative lack of proactivity may have a profound impact on both the general population and South Asian migrants who, because of higher stroke risk, do not enjoy the advantage of a more leisurely approach to stroke management.

1.4.9 Prevalence of cardiovascular risk profile in South Asian stroke populations

Hypertension, diabetes mellitus, smoking and atrial fibrillation (AF) are the most common known conventional risk factors for stroke (Bamford, et al., 1990; Sacco, et al., 2001). The prevalence of these risk factors is known to differ among various ethnic groups (Burchfield, et al., 1994; Cruickshank, et al., 1987) and is also associated with manifestations of different stroke sub-types (Zhang, et al. 2006).

South Asian migrants have been reported to a have a high prevalence of central obesity, hyperinsulinaemia, hypertriglyceridaemia and low HDL cholesterol, but less hypercholesterolaemia and general obesity relative to the general UK population (Cruickshank, et al., 1991; Sewdarsen, et al., 1991). These cross-sectional studies highlight the ethnic variations observed in cardiovascular risk factor profiles.

1.4.9.1 Hypertension

Elevated blood pressure is the most significant known risk factor for stroke in terms of strength of association and consistency of findings (Sacco, et al., 2001). The risk of stroke in the presence of hypertension is increased by three-fold (Zhang, et al. 2006). Migrant South Asian populations in the UK are known to have a higher risk of hypertension (Khattar, et al., 2000; Bhopal, et al., 1999; Whitty, et al., 1999). The prevalence of hypertension in the South Asian population has been reported to be in the region of 30%, and is also reported to be significantly higher than in the Caucasian population (Gupta, et al., 2008). Higher prevalence rate of hypertension has also been reported in a comparative South Asian stroke study in the Quatar where rate was more than 70% (Deleu, et al., 2001) (Table 1.7)

Whilst large studies in stroke are lacking, the disproportionately higher CHD mortality rates in South Asians in the UK have been related to hypertension (Khattar, et al., 2000). However, mean blood pressure levels, the use of antihypertensive therapy and secular trends in blood pressure amongst South Asians appear to be comparable with European Caucasian counterparts (Lyratzopoulos, et al., 2005). Longitudinal studies also show that hypertension is a strong risk factor for CHD in Indians living elsewhere (Patel, et al., 2008b). More population based prospective studies are needed to further explore the impact of migration, on hypertension-related morbidity & mortality amongst migrant South Asians.

Table 1.5: Studies comparing stroke types and risk factors amongst South Asians

Author	Year	Methodology	population	Investigated main epidemiological aspect	Key findings
De Silva et al	2001-03	Prospective	Singapore	Types of stroke and risk factor prevalence	75% of SA had hypertension.
Syed et al	2003	Cross sectional	Pakistan	Type of stroke	Ischaemic strokes (lacunar strokes:42.7%,large artery:26.9 and Cardiac:6.1%) were more common than hemorrhagic strokes
Deleu et al	2001	Cross sectional	Qatar	Type of stroke and Risk factors	SA and Arabs had more lacunar strokes (68%), and Arabs had more diabetes and multiple risk factors.
Vohra et al	2000	Retrospective	Pakistan	Type of stroke and Risk factors	Ischaemic strokes were more commoner than haemorrhagic strokes
Moussoutta et al	1997-05	Retrospective	USA	Type of stroke and Risk factors	SA were younger and had higher prevalence of diabetes and intracranial atherothrombotic infarctions but lower prevalence of hyperlipidaemia compared to European Americans
Kaul et al	2000	Retrospective	India	Stroke types, Risk factors,	Patients with diabetes had more lacunar strokes. hypertension was the commonest risk factor
Bourke et al	1997-02	Cross Sectional	UK	Risk Factors and stroke management	Bangladeshi SA had higher prevalence of diabetes compared to EC and lower level of stroke care

UK=United Kingdom, USA=United States of America, SA=South Asian, EC: European Caucasian.

1.4.9.2 Diabetes mellitus

Diabetes is a continuing global epidemic, particularly concentrated within the Indian subcontinent (King, et al., 1998) (Ramachandran, et al., 2001) such that dispersed migrant populations consistently show a major propensity to develop the disease (Dowse, et al., 1990; Ramaiya, et al., 1995). Their diabetes prevalence that is much higher (approx. 10-15%), compared to the general British population (approx. 4%) (Simmons, et al., 1989), and appears to equal rates in some parts of India (Verma, et al., 1985). Amongst resident Indians, rates of diabetes appear to be associated with urbanisation (Ramachandran, et al., 1997), reportedly lower in rural parts of India (Ramachandran, et al., 1992). However, a direct comparison of Indian migrants to rural contemporaries in villages of origin in India showed no differences in the rates of glucose intolerance (Patel, et al., 2006). Moreover, despite varied lifestyle approaches, cross-sectional studies from Fiji (Collins, et al., 1996) and Tanzania (Seedat, et al., 1990), also question whether urbanisation has a role in explaining the high prevalence of diabetes amongst Indian populations. Hence, not only is diabetes more common amongst South Asians compared to other ethnic groups (Patel, et al., 2007a), but it is likely that this is an inherited risk factor in this population (Moussouttas, et al., 2006).

The prevalence of diabetes is much higher among stroke sufferers and is the second most common risk factor in South Asian stroke patients (Kaul, et al., 2000; Delu et al., 2001). Glycaemia is associated with poorer clinical outcomes in patients with acute ischaemic stroke (Baird, et al., 2003). Increased prevalence of diabetes amongst South Asian patients with stroke may explain their poorer survival, in-particular associated with underlying hypertension (Khattar, et al., 2000). While the presence of diabetes calls for a more stringent management of blood pressure, the underlying pathophysiology that accelerates the risk of stroke is not clear. Moreover, the magnitude of cerebrovascular

risk from diabetes may differ between migrant and non-migrant populations of South Asians, especially amongst those living in the UK.

1.4.9.3 Atrial fibrillation

The prevalence of atrial fibrillation (AF) in stroke patients ranges from 10-29% in population based studies (OXVASC-16%, France Dijon -29%, Manhattan-19%, West Birmingham-10%, South London-21%) (Tolonen, et al., 2002). Conway et al. (2003) reported a lower prevalence of AF in the SA population compared to that of Caucasians (11.8% vs. 34.6%). Compared to other risk factors, the prevalence of AF in South Asians has remained lower and has been shown to have minimal impact on the stroke outcomes. (Conway, et al., 2003). However, the reasons for this observed disparity have not been fully explained and warrant further investigation.

1.4.9.4 Dyslipidaemia

Hyperlipidaemia is a risk factor, which is present in more than 25% of stroke patients (Feigin, et al., 2003). However, the association between hypercholesterolemia and stroke is not clear and one meta-analysis of 45 prospective cohort studies reported no significant association between total cholesterol and ischaemic stroke (Prospective Studies Collaboration Group 1995), although a trend was evident. Trials of LDL cholesterol lowering with statins however report a 20% relative risk reduction for stroke (Libby, et al., 2004).

South Asians are known to have an atherogenic lipid profile, which includes raised triglycerides, low HDL cholesterol and raised Lipoprotein (a) levels (Sewdarsen, et al., 1991). In two South Asian stroke studies, more than 50% of the stroke patients had elevated total cholesterol levels (Tuomilehto, et al., 1993; Deleu, et al., 2006). In Caucasians, the prevalence of hyperlipidaemia ranges from 28.7% (in Dijon, France) to 29.5% (in Oxford) and 32% (in Manhattan, USA) (Feigin, et al., 2003). A similar trend

has been reported in South Asians in the United Arab Emirates, where hyperlipidaemia has become the second most modifiable risk factor after hypertension (Deleu, et al., 2006). However, low HDL levels in South Asians may be more important than higher cholesterol levels in the aetiology of CVD (Sewdarsen, et al., 1991).

In a study by Sharobeem et al. (2007), South Asian stroke survivors were found to have significantly higher apolipoprotein B to AI ratios, and higher Lipoprotein (a) compared with ethnically matched healthy controls. This study highlights the importance of dyslipidaemic management in the treatment and prevention of stroke in a multi-ethnic population, even where the underlying basis for disease aetiology appears to be different.

Other than the mentioned major risk factors, coronary heart disease, smoking and heart failure are also important comorbidities in stroke (Bamford, et al., 1990). South Asians in the UK are more prone to premature, more extensive CHD which also carries a poorer prognosis (Patel, et al., 2008a). There are limited data on heart failure and increased risk of stroke in South Asians (Singh and Gupta, 2005). In one case controlled study by Deleu et al. (2006), 19.6% of the South Asian stroke patients had regional wall motion abnormalities but the mean ejection fraction was more than 60% and was not significantly different to the compared Arab population. The prevalence of heart failure appears to be lower in South Asians compared to recently reported studies from Western populations (Divani, et al., 2009). There are very limited data however, which demonstrate an association between heart failure and increased stroke burden independent of underlying risk factors amongst this population.

Lifestyle factors such as smoking and tobacco use are important in ischaemic strokes (Moulton, et al.,1991). The overall rate of daily cigarette/bidi smoking observed amongst South Asians range from 9-20% where very higher rates are observed amongst Bangladeshi male populations (Glenn, et al., 2009). One population based prospective

study conducted by Bhatacharya et al. (2005) demonstrated an adverse impact of smoking on stroke incidence amongst the South Asian population independent of other conventional risk factors. Recent evidence has supported the role of novel risk factors in determining stroke aetiology. For example, elevated Lipoprotein (a), higher C-reactive protein (CRP) and homocysteine (HCY) concentrations which are particularly raised amongst migrant South Asians are believed to further enhance the risk of developing CVD (Libby, et al., 2004; Tan, et al., 2002).

1.4.10 Conclusions

In general, the incidence of stroke appears to be higher in the South Asians than European Caucasians and has increased exponentially over the last five decades. Comparable rates of stroke prevalence may indicate higher stroke mortality consequent upon disease severity and other co-morbidities. However, studies from India as well as the UK indicate a significant heterogeneity of stroke epidemiology even amongst the South Asian population. In India, for example, there is a perceptible rural-urban gradient in stroke prevalence which can be partly explained by adverse socio–economic circumstances, dietary and lifestyle habits which have been transformed particularly during last two decades. There are no reported migration-related studies to examine the impact of migration on stroke incidence in South Asians particularly those living in Western Europe.

The prevalence of haemorrhagic stroke among South Asians appears to be higher when compared to European Caucasians. This may be intimately associated with higher prevalence of hypertension. However, the increased prevalence of lacunar strokes appears to suggest a greater prevalence of small vessel disease in South Asians, which may be a consequence of abnormal metabolic and glycaemic processes.

Based on the available literature, SA stroke survivors appear to have an aberrant cardiovascular profile compared to EC. Diabetes and hypertension are the most prevalent risk factors in SA stroke survivors. The clustering of these risk factors and their effect on vessel wall characteristics in South Asians may contribute to their higher burden of disease and differing manifestations of stroke in this population. The work with in this thesis is to explore and explain some of these possible pathophysiological mechanisms.

There are also limited data examining the differences of stroke management amongst South Asian stroke survivors, but some support the existence of inequalities in the standard of care available to the South Asian population in the UK. However, the existence of barriers to communication, and other issues rather than ethnicity *per se* may serve to obfuscate the cause of lower standards of care among South Asians. However poor study designs, small sample sizes, poor case ascertainment methods, recording and publication biases and lack of quality in data presentation in the above discussed published studies may restrict critical appraisal, interpretation and comparison of stroke rates between countries as well as amongst ethnic groups.

1.5 Pathophysiology of stroke amongst South Asians

1.5.1 Pathophysiology of ischaemic stroke

Cerebral perfusion can be compromised by several pathophysiological conditions that are associated with atherosclerosis of the extra and intra-cranial blood vessels (Capron, et al., 1988; Prati, et al., 1992). Endothelial dysfunction, increased vessel wall stiffness and inflammation are some of many factors known to play a significant role in the pathogenesis of ischaemic stroke (Febbraio, et al., 2001).

1.5.2 Endothelial dysfunction and Arterial stiffness

The endothelium is a thin monocellular layer that covers all the inner surface of the blood vessels. The endothelium regulates homeostasis by controlling the production of prothrombotic and antithrombotic components, activation of cytokines (angiotensin II, endothelin-1) and modulates vascular tone by effecting smooth muscle cell relaxation (NO mediated) or contraction to cause vasodilatation or vasoconstriction (Furchgott et al., 1980). Cardiovascular risk factors (Sorensen et al., 1994; Celermajer et al., 1993; Williams et al., 1996), inflammation, arterial pressure changes, sheer stress and oxidative stress (Ross et al., 1999; DeGraba, et al., 1992) are believed to alter the endothelial cell's capacity to maintain homeostasis and vascular tone and leads to the so-called endothelial "dysfunction", predisposing development of atherosclerosis.

Endothelial dysfunction is considered a key stage in the development of atherosclerosis initiating luminal narrowing and ischaemia in coronary and cerebral vascular beds (Quyyumi et al., 1997; Cai, 2000; Al Suwaidi, et al., 2000; Halcox, et al., 2002; Schächinger, et al., 2000). It is independently associated with cardiovascular events (Yeboah et al., 2007) and has been shown to be predictive of developing future stroke in Caucasians (Holmes, et al., 2003). However, there are limited data available on its utility in determining stroke outcomes and its prognostic value in South Asians stroke survivors.

The extent of the endothelial dysfunction appears to correlate with conventional risk factor burden, although there is considerable variation in the magnitude of dysfunction observed in individuals with similar risk factor profiles (Quyyumi, et al., 1998). Previous in-vitro and in-vivo studies using different invasive methods support the contention that persons with diabetes (Williams et al., 1996; Clarkson, et al., 1996), hypertension (Panza, et al., 1993) and hypercholesteremia (Sorensen et al., 1994;

Chowienczyk, et al., 1992), appear to have impaired endothelial function. Novel risk factors such as lipoprotein (a), hyperhomocystinemia (both risk factors for stroke), and genetic and ethnic heterogeneity presumably account for some of this variability.

Endothelial dysfunction leads to further damage to physical properties of the arterial media which contains smooth muscle cells, elastin and collagen, and the eventual loss of arterial elasticity (Halcox et al., 2009), compliance and distensibility - leading to increased arterial stiffness. Increased arterial stiffness has been directly associated with increased risk of ischaemic strokes (Blankenhorn, et al., 1994; Bots, et al., 1997) as well as all cause and cardiovascular mortality (Schroeder, et al., 1999). Also, Laurent et al demonstrated the increased risk of fatal outcomes in acute ischaemic stroke with the presence of increased arterial stiffness (Laurent, et al., 2001).

Abnormal arterial stiffness has been demonstrated in people with diabetes and hypertension (Woodman, et al., 2003; Megnien, et al., 1992; Salomaa, et al., 1995). In addition, large arterial stiffness may also increase with hyperlipidaemia (Wilkinson, et al., 2002), inflammation and oxidative stress (Toikka, et al., 1999). However, there is little evidence available on the effects of these risk factors on small artery stiffness and associated stroke and cardiovascular risk (Brooks, et al., 1999; Hassan, et al., 2003) in South Asians especially in relation to strokes due to intra-cranial small vessel disease.

Endothelial function in humans is measured by many techniques (Donald et al., 2006; Gerhard-Herman, et al., 2002) including assessment of the vasodilator response to sheer stress (Doshi et al., 2001). Invasive methods include using intra-arterially administered agents such as, acetylcholine (Furchgott et al., 1980) or salbutamol (Dawes, et al., 1997), the action of such drugs being dependent on the ability of the endothelium to release vasodilator mediators such as nitric oxide (NO) (Joannides et al.,1995; Ignarro et al., 1987). The gold standard non invasive method to measure endothelial function is

based on the principle of reactive hyperemia (FMD)(Doshi et al., 2001). Many methods to assess endothelial function can be invasive, time consuming and difficult to perform (Donald et al., 2006; Barac, et al., 2007). Studies have confirmed that endothelial dysfunction and arterial stiffness can also be assessed by using non invasive techniques (Wilkinson et al., 2002; Donald et al., 2006). These methods have been used as surrogate markers for arterial damage (Deanfield et al., 2005). Detailed accounts of these non invasive methods are described in a different section below.

1.5.3 Impact of conventional risk factor burden on abnormal vessel wall characteristics

Persons with established CVD risk factors such as diabetes (Williams et al., 1996; Calver, et al., 1992) and hypertension (Perticone, et al., 2001) are known to have impaired endothelial function and carry a greater CVD risk. Diabetes and hypertensive disease are both associated with accelerated atherosclerosis as well as age-related arteriosclerosis or 'vascular ageing' (Cruickshank, et al., 2002; Laurent, et al., 1994). The latter are known to cause structural changes in vessel wall characteristics (Oliver, et al., 2003). Indeed, the actions of early initiators of atherosclerosis on the vessel wall are manifested as increased arterial stiffness (O'Rourke, et al., 1999).

Glycaemic status has a profound impact on the endothelium in both microvascular and macrovascular beds (Guerci, et al., 2001). Indeed, diabetes accounts for the majority of excess mortality and morbidity relating to athermatoous disease (Shang et al., 2010). Glycaemic status also has an impact on endothelium-derived Nitric Oxide (NO) production (Calver, et al.,1992), causing a blunted smooth muscle relaxation response in small to medium-sized vessel walls with a consequent increase in vessel tone, thus contributing to increased arterial stiffness (Chowienczyk, et al.,1999). In the presence of diabetes NADPH oxidase production in the vascular beds are increased, thereby enhancing the production of reactive oxygen species and the related oxidative

stress (Föstermann, et al., 2006). These factors are known to increase NO catabolism, impair cGMP activity and consequent endothelial damage (Münzel, et al., 2005).

The principal physiological factors that contribute to increased arterial stiffness other than age include the mean arterial blood pressure (Bramwell, et al., 1922; Armentano, et al., 1995), which is defined as the average pressure throughout the cardiac cycle (Oblouck, et al., 1987). The mean arterial pressure contributes to vascular changes independent of age and probably has a greater impact on the small-to-medium size muscular arteries, leading to higher peripheral vascular resistance.

Despite comparable mean arterial pressure levels , antihypertensive therapy usage (Bhopal, et al., 2002) and changing trends in blood pressure (Lyratzopoulos, et al., 2005), there is still a markedly greater burden of cerebrovascular and cardiovascular morbidity from hypertension in South Asians in the UK compared to the general population (Patel, et al., 2008b). Migration from the Indian subcontinent to the UK and residence therein, may confer an adverse risk exposure to blood pressure in this group (Lane, et al., 2001). The pathophysiological basis for this disparity, however, has not been explained (Agyemang, et al., 2007).

1.5.4 Role of endothelial-derived Nitric Oxide (NO) in the regulation of arterial wall characteristics

People with impaired endothelial function are known to have decreased nitric oxide production. Endothelium-derived nitric oxide (Palmer, et al.,1987) is synthesized from the substrate L-arginine via endothelial Nitric Oxide Synthase (eNOS) (Föstermann, et al., 1994;Busse,et al.,1998; Fleming, et al., 2004) and it exerts its cellular mechanisms via cyclic Guanylate Mono Phosphate (cGMP) (Bauersachs, et al., 1998) second messenger interaction (Melichar, et al., 2004). The NO plays an essential role in maintaining vascular tone, blood pressure (Ohashi, et al., 1998), and lower levels are

thought to be involved in atherogenesis (Maxwell et al., 1998; Wolf, et al., 1997). In addition vascular protective mechanisms of NO include the reticence of platelet aggregation, leukocyte adhesion (Kubes P, et al., 1991), and smooth muscle cell proliferation (Garg, et al., 1989). Given that the majority of the endothelium-derived NO production depends on eNOS concentration, reducing the availability of eNOS also exerts similar impact on the vascular homeostatic mechanisms (Feng, et al., 2002). The dysfunctional eNOS-NO pathway is considered an important early indicator of cardiovascular risk amongst higher risk populations with both prognostic and diagnostic implications (Hare, 2004). Patients with impaired endothelial function are known to have increased risk of cardiovascular disease (Halcox et al., 2002; Rossi, et al., 2003) including strokes (Lip et al., 2002; Corrado, et al., 2008) and reduced NO bioavailability and eNOS expression has been well demonstrated with many other stroke populations (Cheng, et al., 2008; Kim, et al., 2007).

However, there are very limited data examining the molecular interactions of endothelial-derived nitric oxide production amongst the South Asian stroke population investigating the influence on the vessel wall characteristics.

1.5.5 Role of inflammation and oxidative stress in the pathogenesis of stroke and vascular function

Chronic sub-clinical inflammation is now recognized to play a central role in the pathogenesis of CVD and stroke (Rost, et al. 2001). Specifically, C-reactive protein (CRP) is an inflammatory component involved in the atherogenesis (Montaner, et al., 2003). Several studies have also shown that CRP is predictive of stroke. (Di Napoli, et al., 2001) and myocardial infarction (Arici, et al., 2001; Chung, et al., 2001). Interleukin 6 (IL6) and Tumour Necrosis Factor (TNF alpha), are also correlated with the development of CVD (Pradhan, et al., 2002).

Systemic inflammation measured by CRP plays a major role in all stages of athero-thrombosis and provides a critical pathophysiological link between plaque formation and acute rupture, leading to occlusion and infarction (Lhermitte, et al., 1968). The degree of inflammation could vary in different vascular beds in the cerebral blood supply where atherosclerotic process in the intracranial arteries may have differential characteristics to that of extra-cranial large arteries (Lammie, et al., 1999). Furthermore the degree of inflammation indicated by elevated markers of inflammation (Kalra, et al., 2005) and other metabolic factors such as oxidative stress and glycaemic status could be different amongst ethnic groups. However there is very limited data on its role in the development of stroke and its predictive value in predicting stroke outcomes in South Asians (Somani, et al., 2006).

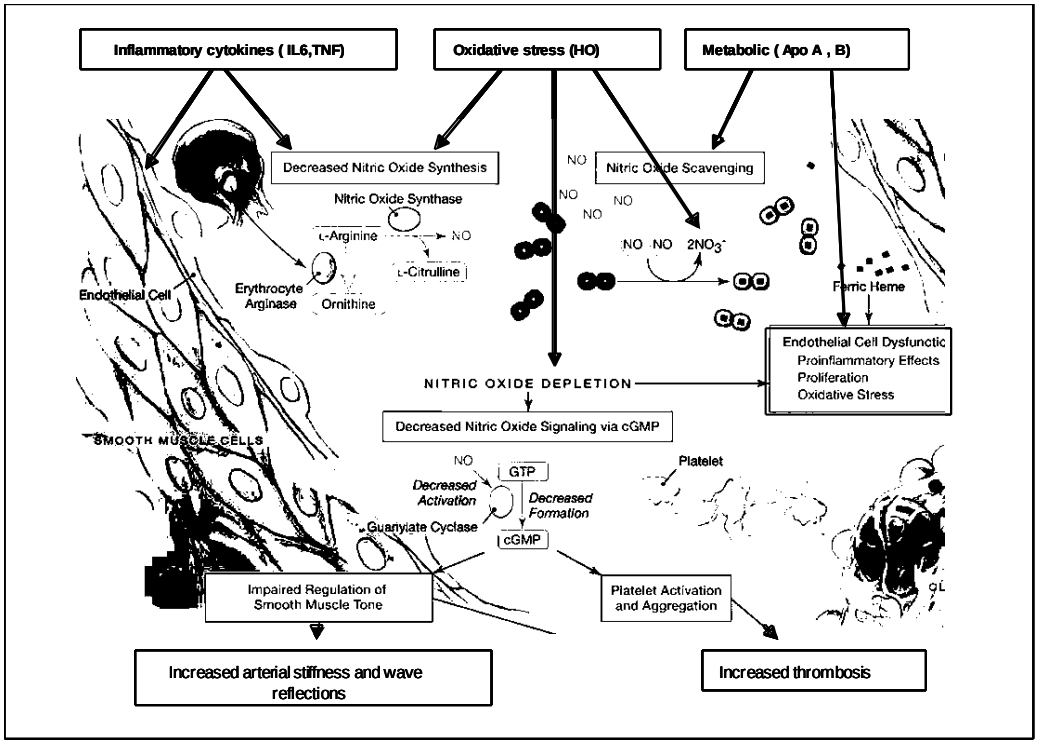
Heme-oxygenase (HO 1) is an inducible protein which acts as a rate-limiting enzyme that catalyzes the degradation of heme into biliverdin, iron and carbon monoxide (CO). These metabolites are known to have a protective action against oxidative stress-mediated injury. Increased levels of expression of HO 1 have been reported in many cardiovascular conditions (Duckers, et al., 2001; Kapturczak, et al., 2004) but there are very limited data available in relation to cerebrovascular disease. It is postulated to have a role in modulating the vascular tone in pathological conditions such as ischaemia (Duckers, et al., 2001). Ameriso et al (2005) demonstrated a higher level of expression of HO 1 in patients with carotid atherosclerosis. However its prognostic value and clinical relevance of use of HO in South Asian stroke patients have not been previously investigated.

1.5.6 Ethnic differences in pathophysiology of stroke amongst South Asians

Ethnic variations in arterial stiffness, endothelial damage and inflammation are known to exist (Young, et al., 2007) but there are very limited data available on

pathophysiological differences in arterial stiffness (Din, et al., 2006), endothelial damage and inflammation in South Asian stroke survivors. In addition, its relationship to stroke and disease outcomes have been hampered by lack of population based studies amongst South Asian stroke survivors. Available literature on healthy South Asians suggests that they have an excess risk of cardiovascular disease associated with increased arterial stiffness (Din, et al., 2006) and endothelial damage (Raji, et al., 2004) compared to that of Caucasians. It is evident that south Asians are more susceptible to small vessel disease and to acquiring lacunar type of strokes (Deleu, et al., 2006). However, it is not clear whether this can be totally explained by the known ethnic differences in traditional CVD risk factors. Therefore, it is reasonable to assume that proposed differences in arterial stiffness and endothelial damage along with clustering effect of other aberrant metabolic, inflammatory, eNOS-NO axis parameters may account for this disparity (Figure 1.3).

Figure 1.3: Proposed underlying pathophysiological mechanisms contributing to abnormal vessel wall characteristics amongst South Asians.



*Adopted and modified from: http://www.google.co.uk/search?hl= m&xhr=t&q=endothelial+function: accessed on 8th January 2008.

Measurements of indices of arterial stiffness and endothelial dysfunction in South Asian stroke survivors should aid better cardiovascular risk stratification amongst South Asians and enable clinicians to instigate a more sensitive and holistic approach towards their risk factor control. This thesis intends to examine the differences in prevalence of cardiovascular risk profile and related underline pathophysiological processes in South Asian stroke survivors. The next section looks at the current evidence for any association between arterial stiffness and ethnicity to aid accurate interpretation of the findings of the pathophysiological studies within this thesis work.

1.6 Arterial stiffness and ethnicity

1.6.1 Introduction:

Current evidence suggests an association between arterial stiffness, ethnicity and underlying genetic polymorphisms (Kingwell, et al., 2007). Other recent studies serve to support the hypothesis that arterial stiffness is, in part, an inherited and dynamic trait and the molecular pathways regulating arterial stiffness are not completely understood (Yasmin, et al., 2006). This section explores measures of arterial stiffness among different ethnic groups including South Asians.

1.6.2 Methods: literature review strategy

A MEDLINE and EMBASE search was performed using the terms (MeSh) : (Ethnicity) OR (South Asian, Indian, Sri Lankan, Bangladeshi, Pakistani, European Caucasian, African, Afro-Caribbean, Chinese, Japanese, Hispanic), AND (Arterial stiffness) OR (Pulse Wave Velocity (PWV)), Augmentation Index (AI), Stiffness Index (SI), Pulse Pressure (PP)). For the purpose of this review, 17 representative studies were selected, based upon 15 cross sectional and two prospective studies describing ethnic variation in arterial stiffness. We did not perform a meta-analysis or statistical calculations to combine or analyze the data, and only reviewed and analyzed

descriptively, because of the methodological heterogeneity of measurements of arterial stiffness and differing study designs.

1.6.3 Arterial stiffness and Ethnicity

The majority of US-based studies compare ethnic variations in indices of arterial stiffness between White and Black American populations (Table 1.8). A genetic twin study by Young et al. (2007) examined the heritability of arterial stiffness (measured as PWV) and its dependence on ethnicity. This study showed no relationship between ethnicity or gender and the degree of arterial stiffness but indicated substantial heritability. Furthermore, more than 25% of this heritability was explained by genetic makeup. Hlaing et al. (2006a) also found no differences in arterial stiffness between White Americans and Afro-Caribbean Americans. Both these studies sampled a younger (20-25 years) and healthier population compared to other published studies by Chen et al., (2006) and Din-Dzietham et al. (2004), both of which demonstrated significantly higher arterial stiffness in a middle-aged Afro-Caribbean population which also had other established cardiovascular risk factors. Similarly, a study by Lemogoum et al. (2006) demonstrated increased arterial stiffness in response to smoking in African-Americans.

Two large population-based studies (the Bogalusa Heart Study (2006) and the ARIC Study (2004)) used PWV to measure arterial stiffness and both showed greater PWV values in non-Whites when compared to Whites, whilst MAP and lipid abnormalities predicted increased arterial stiffness. In the UK, Kalra et al. (2005) compared indices of arterial stiffness amongst healthy age- and gender-matched Afro-Caribbeans and European Caucasians and demonstrated no ethnic difference in the measurement of arterial stiffness. In contrast, Chaturvedi et al. (2004) compared high-risk populations and demonstrated that Afro-Caribbean persons with diabetes had increased indices of arterial stiffness compared to age- and gender-matched diabetic

American Caucasians. This study demonstrated a disproportional impact of glycaemic status on vessel wall characteristics, leading to higher arterial stiffness and an increase in cardiovascular risk amongst Afro-Caribbeans.

Other studies have revealed further inconsistencies in the measurement of arterial stiffness amongst Chinese, Japanese and Russian populations. Nonetheless, Foo et al. (2007) showed comparable arterial stiffness measurements between Singaporean Chinese and Caucasian ethnic groups. Indices of arterial stiffness were significantly higher in those Chinese who recently migrated to Australia compared to those Chinese who migrated some decades ago (Dart, et al., 1995).

Table 1.8: Ethnicity related studies on arterial stiffness (I)

Study	Year	Design and Number	Arterial stiffness measurement method	Ethnic groups compared	Main outcome
Young et al.	2007	Healthy population Twin Study,Cross sectional (n=702), Age 12-30 years	Aorto radial and aorto dorsalis pedis Pulse wave velocity	Blacks Americans and American Caucasians	Individual differences in the arterial stiffness was substantially heritable, and >25% of this heritability is explained by genes. No ethnic or gender differences
Foo et al.	2007	Healthy population,Cross sectional (n=67), Age < 12 years	Pulse transit time	Singapore Caucasians and Singapore Chinese	Arterial stiffness between two groups were not significantly different
Heffernan et al.	2007	Healthy population,Cross sectional (n=24), Age 21-23 years	Carotid femoral and femoral dorsalis pedis pulse wave velocity	African-Americans and American Caucasians	African American men had significantly higher resting aortic stiffness.
Brown et al.	2007	Population with risk factors Cross sectional (n=283), Age 30-62 years	Augmentation Index and Carotid femoral pulse wave velocity	Remote Indigenous and European Australians	Indigenous Australians have higher indices of peripheral and central arterial stiffness than European Australians of similar 35% of the risk was not explained by traditional CVD risk factors
Hlaing et al.	2006	Healthy population, Cross sectional (n= 491) , Age 20-22 years	Arterial pulse pressure	American Caucasians, ,Hispanic and Black Americans	No variation between ethnic groups. Male had higher arterial stiffness
Chaturvedi et al.	2006	Population with diabetes mellitus, Cross sectional (n=303), Age 40 to 65 years	Carotid femoral pulse wave velocity	African Caribbean's and European Caucasians	African Caribbean's with diabetes had increased stiffness compared to Europeans
Chen et al.	2006	General population, Cross sectional, (Bogalusa Heart study), (n=900) , Age 24-43 years	Carotid femoral Pulse wave velocity	African-Americans and American Caucasians	African-Americans had higher pulse wave velocity. However ethnicity didn't independently associated with PWV but with systolic blood pressure
Hlaing et al.	2006	Healthy population Prospective study (9 years) (n=487). Age 7 to 16 years	Arterial pulse pressure	African-Americans and American Caucasians	African-Americans who were before the age of 10 years had increased arterial stiffness

Table 1.9: Ethnicity related arterial stiffness studies (II)

Study	Year	Design and Number	Arterial stiffness measurement method	Ethnic groups compared	Main outcome
Liu et al.	2005	Healthy population, Cross sectional study , (n=475) Age 40-60 years	Brachial pulse wave velocity	Japanese and Russians	Russians had higher arterial stiffness compared to Japanese at a younger age
Kalra et al.	2005	Healthy population,Cross sectional (n=164), Age 30-75 years	Stiffness index and Carotid intimal medial thickness	Afro-Caribbean and European Caucasians	Afro-Caribbean's had comparable Stiffness index to European Caucasians but had higher carotid intimal medial thickness
Chaturvedi et al.	2004	Population with risk factors, Cross sectional (n=202), Age 40-64 years	Carotid femoral and radial pulse wave velocity	Afro-Caribbean and European Caucasians	African Caribbean's had increased Carotid femoral pulse wave velocity but not Carotid radial pulse wave velocity
Chen et al.	2006	Healthy population, Cross sectional (n=403), Age 25-37 years	Common carotid artery Petersons and Youngs elstic modulus	African Americans and American Caucasians	African Americans had higher arterial stiffness associated with lower eNOS G894T polymorphism
Din-Dzietham et al.	2004	Healthy population Prospective study (3 years) (n=2727), Age 45-64 years	Mean beta stiffness index (Echo tracking)	African Americans and American Caucasians	African Americans had higher arterial stiffness particularly at early age
Ferreiraet al.	1999	(Hypertensive population) Cross sectional (n=120) Age 40-60 years	Carotid femoral pulse wave velocity	African Americans and American Caucasians	African Americans with hypertension had increased arterial stiffness
Dart et al.	1995	Healthy population, Cross sectional (n=83)	Aortic pulse wave velocity and the aortic elastic modulus	migrants Chinese and Australian Chinese inhabitants	Migrant Chinese had increased arterial stiffness and duration of Australian residence was associated with increase stiffness.

In this same study, increasing duration of Australian residence appears to be accompanied by an increase in arterial stiffness associated with dietary and lifestyle differences but without accompanying differences in cardiovascular risk. Also, cardiovascular mortality rates in Russia have become the highest in the world (Davydov, et al., 2007), whilst amongst the Japanese, mortality rates have remained at relatively lower level. In a comparative study, Liu et al. (2005) demonstrated increased PWV in apparently healthy Russian population compared to age and gender matched Japanese population, possibly providing a pathophysiological basis for their higher cardiovascular risk.

Based on available evidence, complex disparities (related in part to ethnicity) exist concerning arterial stiffness, especially in studies from developed countries, which principally compare Afro-Caribbean and Caucasian groups.

The available literature however does reveal similar disparities in arterial stiffness amongst Afro-Caribbean groups who are older and have established risk factors. Comparable degrees of arterial stiffness in younger, healthier populations and differences in age and gender-matched older populations hints at the disproportionate impact of conventional cardiovascular risk factors on vessel wall characteristics which are cumulative and become manifest later in life. This may indicate an adverse genetic susceptibility of certain ethnic groups, which is responsible for increased arterial stiffness and consequently, a higher cardiovascular risk. However, the majority of the studies demonstrate lack of robust study designs comprising elements of functional genomics, larger sample sizes, and longitudinal approach. More importantly, methods of patient recruitment, study designs and studied age groups were variable, demonstrating a significant methodological heterogeneity. There also appears to have been a variation in

the measurements and methods used to measure different indices of arterial stiffness amongst different ethnic groups.

1.6.4 Conclusion

Current evidence suggests an association between arterial stiffness and ethnicity. There appears to have been a variation in the measurement of different indices of arterial stiffness amongst different ethnic groups such as in Afro-Carribeans. Research undertaken to date has not been entirely successful in explicating the underlying causative mechanisms of increased arterial stiffness in certain ethnic groups. In particular, there are very few studies involving higher CVD risk ethnic groups such as Asians who are likely to have abnormal indices of arterial stiffness. There are no studies examining vascular structure and function in South Asian stroke survivors. More comparative studies are needed to comprehensively examine the arterial structure and function amongst South Asians. The findings of this review further highlight the paucity of pathophysiological studies amongst South Asian stroke survivors and provide a firm basis for the proposed studies within this thesis work.

Chapter 2 : General Methodology

2.1 Study Rationale

Preceding chapter highlights the evidence for higher rates of ischaemic strokes and related stroke mortality amongst South Asians compared to European Caucasians. What is the reason for this observed disparity? Based on the available evidence (Chapter 1) a pathophysiological explanation for this ethnic variation in disease rates is not apparent.

In the UK, Coronary Artery Disease (CAD) amongst South Asian migrants is due to both hypertension and diabetes (Chapter 1.3), suggesting that an aberrant cardiovascular risk profile may in part underlie the increased risk of stroke in this population. As discussed in the former sections (Chapter 1.5), diabetes, hypertension, inflammation and oxidative stress can have a profound impact on the endothelium in both microvascular and macrovascular beds by altering the intra-cellular metabolic processes. Dysfunctional endothelium has a direct influence on endothelium-derived Nitric Oxide (NO) production, causing a blunted smooth muscle relaxation response in vessel walls with a consequent increase in vessel tone, thus contributing to increased arterial stiffness (Figure 1.3). Literature on other ethnic groups indicates an association between increased arterial stiffness and ischaemic stroke mortality (Chapter 1.6).

Therefore, as a step towards understanding the pathophysiology of cerebrovascular disease amongst this higher risk population, my thesis intends to examine the differences in prevalence of cardiovascular risk profile and vessel wall characteristics, specifically examining the arterial stiffness, endothelial function and related: metabolic, inflammatory and oxidative stress indices amongst South Asian stroke survivors.

2.2 Main hypotheses & Study outline

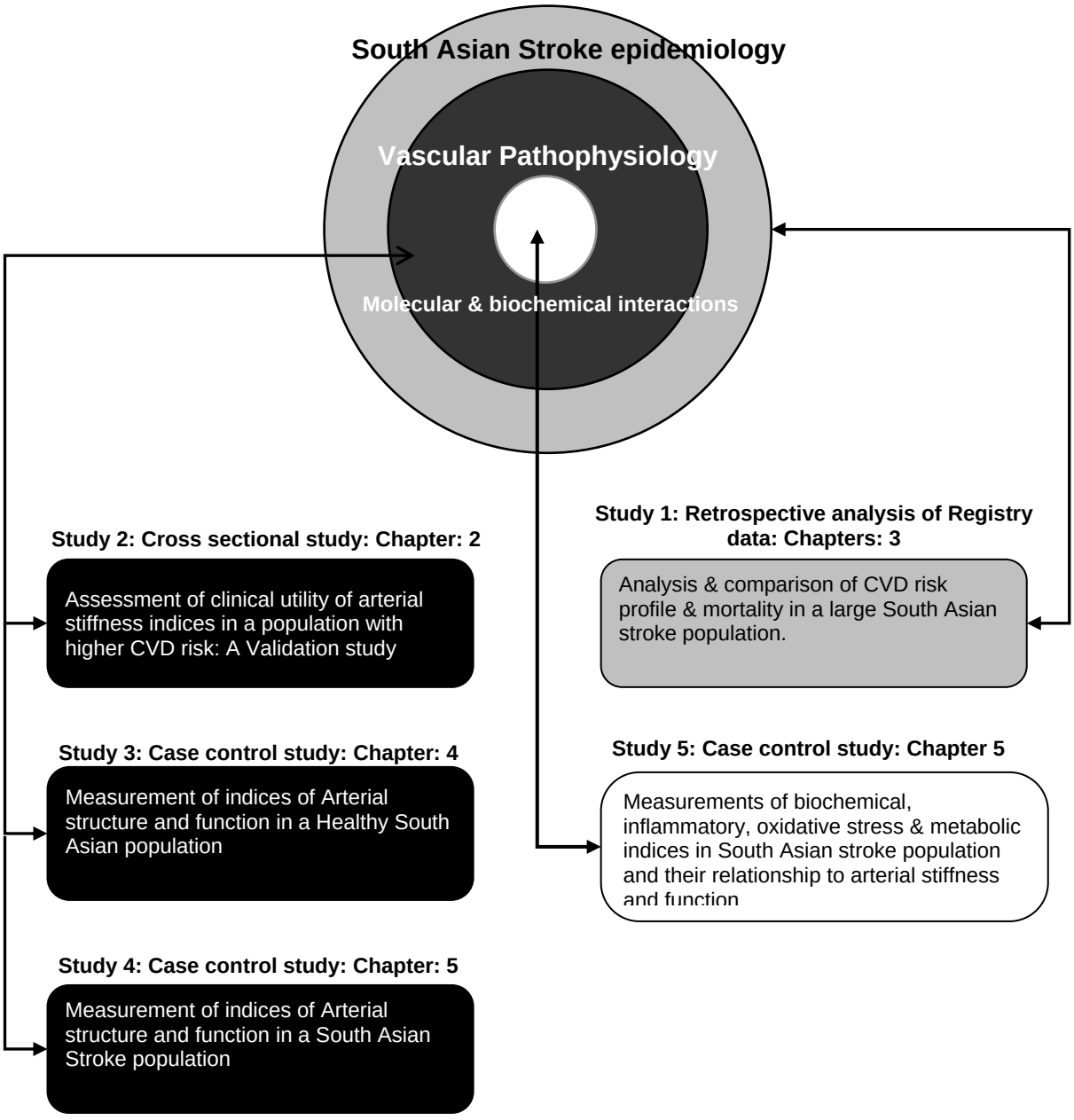
Hypothesis 1

The cardiovascular risk profile in South Asian stroke survivors differs to counterparts in the general population, and these differences may explain variations in mortality.

Hypothesis 2

Arterial stiffness and indices of endothelial dysfunction are higher amongst South Asians stroke patients compared to Caucasian counterparts, and differences in CVD risk profile and related biochemical (Nitrate, eNOS and cGMP) , inflammatory (Interleukin (IL6), High sensitivity C-reactive protein (hsCRP), Tumour necrosis factor (TNF alpha)), metabolic (Apo lipo protein (Apo A), Apo lipo protein B(Apo B)), and oxidative stress (oxidised Low Density Lipo-protein (oxLDL), Haem-Oxygenase (HO)) abnormalities may explain these aberrancies.

Study Outline



2.3 Aims & Objectives:

Study 1: Retrospective analysis of Registry data (Chapter 3):

Literature appraisal (Chapter 1) indicates a significant paucity of comparative cardiovascular risk profile data in South Asian stroke survivors. Therefore, as a step towards understanding the pathophysiology of cerebrovascular disease amongst this higher risk population, the objective of this study was to determine the differences in the prevalence of cardiovascular co-morbidities and their association with mortality compared to other ethnic populations in the UK by retrospectively analysing registry data of a larger stroke population in West Midlands, a home to a larger South Asian migrant population.

Study 2: Validation study (Chapter 2):

Pathophysiological understanding of stroke and CHD is closely allied with accelerated atherosclerosis and age-related arteriosclerosis which are known to alter vessel wall characteristics. Measures of arterial stiffness indices are accepted as independent markers of CVD. Arterial stiffness indices derived from Digital Volume Pulse (DVP) is a non invasive indirect technique of measuring arterial stiffness peripherally. The objective was this study was to determine the repeatability & reproducibility of DVP technique, in view of using this technique to assess arterial structure and function in a higher risk South Asian stroke population.

Study 3: Case control study (Chapter 4):

Literature review on arterial stiffness and ethnicity (Chapter 1.6.3) highlighted the significant paucity of available data on indices of arterial structure and function amongst South Asians. According to available evidence, there is a markedly earlier progression of

disease in South Asians, suggesting that the pathogenesis of sub-clinical atherogenesis and premature arteriosclerosis is manifest even in apparently healthy individuals. The principal objective of this study is to firstly determine the structural characteristics of the vessel wall amongst healthy SA compared to that of EC.

Study 4: Case control study (Chapter 5):

The main aim of this study is to understand the underlying vascular pathophysiology of South Asian stroke survivors. Given the higher prevalence of diabetes and hypertension amongst SA compared to EC and adverse impact of these risk factors on endothelium & vessel wall characteristics, the objective of this study is to comprehensively examine the vascular risk indices (arterial stiffness and endothelial function) amongst SA stroke survivors to compared to EC. In addition this study also intends to examine the impact of inflammation, metabolic status and oxidative stress parameters on arterial stiffness and function.

2.4 Study Designs:

2.4.1 Study Design 1: Retrospective analysis of Registry data

A stroke registry was developed using patients' information system and electronic case notes to determine prevalence of cardiovascular risk profile in a multi ethnic population admitted to Sandwell and West Birmingham NHS Trust over a nine-year period. Data from the stroke registry was combined with five years follow up data obtained from the national mortality tracing system to determine events-free survival rates amongst a multi ethnic population admitted to our Sandwell and West Birmingham NHS Trust over a five-year period.

The hospital discharge coding (International Classification of Disease (ICD) (10th revision) was used for the identification of patients admitted with any form of stroke (ICD: 160-169) during the period between 1997 and 2005. Patients with a diagnosis of haemorrhagic strokes (ICD: 160, 161 and 162) and unspecified aetiology (ICD: 164) were excluded from the study cohort and patients with a cerebral infarction (ICD: 163) were selected for the registry. Using information of our local Hospital Activity Analysis Coding register, patient's age at date of admission, sex, ethnic group, and established CVD risk factors (AF [ICD:148], hypertension [ICD: 110-115], diabetes mellitus [E10-14], myocardial infarction [ICD:121], and peripheral vascular disease [ICD:170 and 173] were added to the registry. To minimise any inaccuracies electronic hospital case records, which composed of discharge letters, CT reports and investigation results, were retrieved in the first instance using the patient's hospital number identified from the ICD coding based database. Original paper-based case notes were only retrieved in about 15% of the patients to complete if any missing information. To validate the database and minimise any inaccuracies in coding, in pre-identified 12.4% of the patients (patients who were pre-screened for NIASTAR clinical trial; EudraCT No: 05/Q1606/148) the diagnosis of stroke [ICD 163] were cross-referenced with computerized CT reports and final diagnosis details on the hospital computer system. In addition, 160 of these patients the diagnosis of ischaemic stroke [ICD: 163] were crosschecked with stroke unit data collection sheet final diagnosis details, which is completed by a consultant stroke physician based on clinical and radiological information. Thirty-day case fatality data, based on the all cause mortality, obtained from the National Health Tracing Services was added to this database (NHS strategic tracing service 2006). This ICD coding system has been proved free of errors for definitive diagnoses (George and Maddock, 1979), although errors are found in the coding of non-specific diagnoses such as 'viral infections' or 'gastroenteritis'. (Please see limitation section for details)

Classification of stroke type (Ischaemic / haemorrhagic / unspecified aetiology) was based on the radiological and clinical evidence available for ICD coding at the time of patient discharge or death. Diagnosis of stroke had been defined on clinical symptoms supported by a cerebral imaging (usually a CT scan) in accordance with the WHO criteria for diagnosis of acute stroke (Hatona, et al., 1976). According to our hospital practice, which is based on standard national guidelines(NICE, 2006), each patient admitted with symptoms and signs of stroke will be examined by a senior physician prior to routine blood tests and special radiological investigations (CT/MRI) for the diagnosis of acute stroke and its pathological type within 48 hours. Each patient will undergo at least one ECG recording and twice daily blood pressure measurements. Coding of hypertension based on the defined blood pressure > 140/90mmHg on at least two separate readings. Diabetes diagnosis based on the relevant clinical history and biochemical evidence of at least 02 measurement of Fasting Blood sugar readings > 7.2mmol/l Patients with a total cholesterol level > 5mmol were considered as hyperlipidaemic. Diagnosis of atrial fibrillation depends on the clinical evidence supported by a 12 lead ECG. History of myocardial infarction was based on ECG evidence supported by elevated cardiac troponin levels. Echocardiographic evidence for diagnosis of valve lesions was mandatory to be given a diagnosis of valvular heart disease. Diagnosis of peripheral vascular disease is based on the patient's clinical symptoms and signs, supported by any radiological evidence of vascular abnormalities.

2.4.2 Design 2: Cross sectional/Case control studies

2.4.2.1 Study Population: Healthy & Risk factor controls

Information from the 2001 census revealed that between 20 and 25% of the 300,000 population of Sandwell are from South Asian ethnic minority groups [including Bangladeshi (1.21%), Indian (9.14%) and Pakistani (2.95%) populations]. SA were

defined by self-reported ethnicity as being Indian, Pakistani, Sri Lankan, Nepalese, and Bangladeshi, and whose grandparents (at least three) also originated from the Indian subcontinent (Chapter 1.2).

Volunteers who initially participated in community based cardiovascular risk screening clinics in Sandwell and West Birmingham area (West Midlands, UK) were invited to attend the vascular research clinic. Using a representative sampling approach, volunteers who expressed their willingness to participate in the study and had similar socio-economic backgrounds based on the Townsend deprivation index were recruited in a subsequent visit. The total cohort (aged 30 to 75 years) comprised apparently healthy people without any known established risk factors & people with established risk factors.

All healthy subjects were free from any documented cardiovascular disease or cerebrovascular disease. None of the healthy subjects were on any regularly prescribed cardiovascular medications at the time of the study. This was established by the elicitation of a full medical history and the completion of a comprehensive physical examination performed by a qualified medical practitioner. Any person with abnormal blood pressure measurements (>140/90mmHg), fasting blood sugar level (>7.2 mmol/l) or a total cholesterol level (>5mmol/l) were excluded from the healthy cohort. Demographic data including details of smoking habit and alcohol consumption were collected.

SA risk factor controls were categorised as either having an established diagnosis of hypertension, diabetes, hyperlipidaemia and who were receiving treatment or evidence of abnormal mean blood pressure measurement (>140/90 mmHg), raised fasting plasma glucose (>7.2 mmol/l), raised serum cholesterol (>5mmol/l), according to the Joint British Societies 2 guidelines (JBS 2, 2005) but without any established cerebrovascular disease (past medical history of stroke or transient ischaemic attack).

Patients with a history of severe heart, renal or respiratory failure, established chronic inflammatory or lung disease (e.g. asthma, chronic obstructive airway disease) or ischaemic heart disease (ongoing anginal symptoms, currently on prescribed anti-anginal medications, past medical history of myocardial infarction, coronary artery bypass graft or percutaneous coronary intervention) were excluded from risk factor control group.

2.4.2.2 Study Population: Ischaemic Stoke survivors

South Asian stroke patients with a diagnosis of ischaemic stroke, who were initially admitted to Sandwell and West Birmingham NHS Trust (Sandwell and City Hospitals) between 2005 and 2007, were invited to participate in the study after three months of the initial presentation. Of the responders, the first consecutive 100 patients who attended the initial screening visit were prospectively recruited for the vascular sub study. Based on the power calculations (for vascular measurements) at least 60 people were required in each group. Therefore using the same stroke unit database , age, gender and post code (same area) matched Caucasian stoke patients who were admitted during the same period were invited and of the responders, the first attended 60 patients were recruited for this vascular sub study. After excluding the patients who had died or who developed a second stroke with in the first three months period, 28% of the South Asian and 6% of the Caucasian stoke patients who originally invited and agreed to participate in the study did not attend the subsequent screening visit.

2.4.2.3 Evidence and criteria used for stroke classification

The diagnosis of stroke was established on the basis of clinical symptoms supported by a cerebral imaging (CT scans) in accordance with the WHO criteria for diagnosis of acute stroke (Hatona, et al., 1976). In keeping with local hospital practice, which is based on standard national guidelines (NICE, 2007), each patient admitted with symptoms and signs of stroke was examined by a stroke physician prior to routine blood

tests and radiological investigations (CT) in order to confirm the diagnosis of acute stroke and its pathological type within 72 hours. All CT scans were reported by a neuro-radiologist and each patient's clinical condition, including the quality of life (EQ5D, Glick, et al., 1999), motor function (Modified Rankin Scale (MRS) (Wolfe, et al., 1991) and stroke severity (Scandinavian Neurological Stroke Scale [SNSS]; Côté, et al., 1989) and neurological deficit, was recorded on a standard Performa. Each patient underwent at least one ECG recording and twice daily blood pressure measurements and subsequently had carotid duplex scan imaging. Based on the above information, ischaemic stroke subtypes were classified according to TOAST (Adams, et al., 1991) and Bamford (Bamford et al., 1991) criteria by a qualified stroke physician (Table 2.1).

Table 2.1: Evidence and criteria used for stroke classification

	Clinical evidence	**Radiological evidence**	**Other evidence**
TOAST classification			
Large vessel	Cortical, subcortical, cerebellar or brain stem symptoms and signs (aphasia, apraxia, neglect)	Lesions on CT >1.5cm in cortex, sub cortex or brain stem with compatible symptoms	Carotid duplex:> 50% stenosis.
Small vessel	Signs and symptoms of a lacunar syndrome (pure motor, pure sensory, sensori-motor hemiparesis or dysarthria and clumsy hand syndrome)	Lesions on CT <1.5cm suggestive of lacunar infarctions with compatible symptoms	Absence of large vessel disease or embolic disease
Cardio-embolic	Clinical signs of large vessel disease, AF, Atrial flutter	Lesions on CT >1.5cm in cortex, sub cortex or brain stem with compatible symptoms	ECHO confirmed embolic source
Bamford classification			
TACI	New higher cerebral function dysfunction (dysphasia / dyscalculia /apraxia / neglect /visuospatial problems) plus (Homonymous visual field defect) plus (Ipsilateral motor and/or sensory deficit of at least two areas of face, arm and leg)		
PACI	Two of the three components of TACI, or isolated dysphasia or other cortical dysfunction, or motor/sensory loss.		
POCI	Cranial nerve deficit with contralateral hemiparesis or sensory deficit, or disorders of conjugate eye movement, or isolated cerebellar stroke, or isolated homonymous hemianopia.		
LACI	Pure motor or pure sensory deficit affecting two of three of face, arm, and leg, or sensorimotor stroke or ataxic hemiparesis or dysarthria plus clumsy hand, or acute onset movement disorders.		

(TACI: Total Anterior Circulation Infarct, PACI: Partial Anterior Circulation Infarct, POCI: POsterior Circulation Infarct, LACI:LACunar Infarct)

2.4.3 Ethical Approval & Consent:

This study was approved by the West Birmingham local research Ethics Committee (Appendix 1). Sandwell & West Birmingham NHS Trust Research & Development department evaluated & audited this study and offered a favourable opinion. This theses study proposal was awarded a R&D research grant in the annual Gaisford Research competition in 2007. Written informed consent was obtained from all patients and controls enrolled in the study.

2.5 Measurements

2.5.1 Anthropometric measurements

Following consent, participants were invited to be assessed for the measurement of body weight (Seca floor scales) and height (Leicester height measure, Seca Ltd, Birmingham, UK), where the subject was measured without shoes in light clothing, and advised to stand in a posture to accommodate their heads to be in the vertex plane for height assessment . However, to measure weight amongst the stroke patients who were unable to stand, Seca 958 Digital Chair Scale (UK) was used where height was recorded using a metal tape in the supine position. Body mass index was calculated as BMI = weight (kg) / (height (m))^2

The subject's waist hip ratio was measured using a metal tape. The waist was chosen as the narrowest circumference above the umbilicus and below the ribs of a subject. The hip was measured over clothing (assessed for thickness) and chosen as the widest circumference around the buttocks and was again measured in duplicate (Larson, et al., 1984). The WHR was calculated as follows: WHR= waist girth (cm) / hip girth (cm).

2.5.2 Pathophysiological measurements

2.5.3 Blood pressure measurement

Systolic and diastolic brachial arterial blood pressure levels were measured in both arms at one minute intervals in the first instance and repeated in the arm with the higher blood pressure measurement with the validated semi-automatic Omron HEM-705CP (Omron Healthcare Europe, Mannheim, Germany) using appropriate sized cuffs, after more than five minutes sitting. The mean of the last two blood pressure levels was used in the analysis. Heart rate was recorded from the last recorded blood pressure reading. Mean arterial blood pressure was calculated by adding one-third of the diastolic pressure to the pulse pressure.

2.5.4 Measurements of arterial stiffness

Arterial stiffness can be estimated from the models of the circulation to derive systemic arterial stiffness (Nichols, et al., 2005) or can be directly measured non-invasively, at various sites along the arterial tree (Van Bortel, et al., 2002). A major advantage of the regional and local evaluations of arterial stiffness is that they are based on direct measurements of parameters strongly linked to wall stiffness.

The measurement of PWV is generally accepted as the most simple, non-invasive, robust and reproducible method to determine arterial stiffness (Benetos, et al., 1993). Carotid-femoral PWV is a direct measurement, and it corresponds to the widely accepted propagative model of the arterial system (Latham, et al., 1985). PWV is usually measured using the foot-to-foot velocity method from various waveforms. These are usually obtained, transcutaneously at the right common carotid artery and the right femoral artery (carotid-femoral' PWV), and the time delay or transit time measured between the feet of the two waveforms.

In recent times other different methods have been developed to measure regional arterial stiffness by means of assessing wave form reflections non-invasively (Adji, et al., 2004). Repeatability and reproducibility of these different methods have been evaluated in many cross sectional study designs (O'Rourke, et al., 2002) compared to gold standard PWV technique (Table 2.1). Of these methods, stiffness index measured by digital volume pulse analysis (DVP) technique has been demonstrated to have a good agreement with PWV measurements and can be easily performed in day-to-day clinical practice.

Table 2.2: Comparison of sensitivity and specificity and reproducibility of different methods compared to direct carotid femoral pulse wave velocity (PWV) technique

	Area ROC	Sensitivity (%)	Specificity (%)	CV (%)
Augmentation index	0.99	100	93	11.3
Stiffness index	0.95	87	87	12.3
Central PWV	0.91	73	93	7.6
Central PP	0.78	53	80	15.6
Distal PWV	0.68	67	64	22.4
Brachial PP	0.46	33	60	8.0

PP: Pulse Pressure, PWV: pulse wave velocity. Adapted from Assessment of central and peripheral arterial stiffness.Woodman, et al., (2005) American Journal of Hypertens,18: 249-26

Arterial stiffness was measured using the Digital Volume Pulse analysis (DVP) technique. The Digital Volume Pulse (DVP) analysis method is a non-invasive technique of measuring pulse wave reflections to determine the arterial stiffness peripherally (Millasseau, et al., 2006). Arterial stiffness using the above method has been proven to be a validated, reproducible technique with minimal intra-observer variation (Millasseau, et al., 2006). The Stiffness Index derived from this method has been demonstrated to have a good correlation to PWV (Woodman 2005; Sollinger, et al., 2006; Adji, et al., 2004) whilst the sensitivity and specificity of this technique is comparable to the PWV method in the identification of patients with latent cardiovascular disease (Millasseau, et al., 2002).

2.5.5 Measurement of endothelial function (Δ RI %)

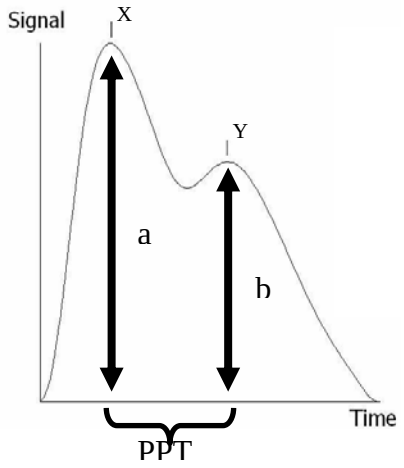
As mentioned previously (Chapter 1) endothelial function in humans can be measured by many techniques, including assessment of the vasodilator response to sheer stress (reactive hyperemia) (Doshi et al., 2001). Many methods to assess endothelial function can be invasive, time consuming and difficult to perform (Donald et al., 2006). However, the relatively novel DVP analysis technique is a non–invasive option, similar to other non-invasive measurements such Flow Mediated Dilatation (FMD) with minimal inter- and intra-observer variation (Millasseau, et al., 2006). This technique is an indirect method of determining endothelial function peripherally (Rambaran, et al., 2008) by measuring the peripheral pulse waveform changes and wave reflections (Hayward et al., 2002) following non-parenteral administration of salbutamol and glyceryl trinitrate (GTN), and has shown initial utility in detecting individuals with impaired endothelial function (Chowienczyk, et al., 1999 and Wolfe, et al., 2002). Compared to other non-invasive methods the potential benefits of this technique are that since measurements are taken at the finger tip, the procedure is considerably easier to perform, less operator-dependent with minimal observer bias. The instrument itself is readily portable and inexpensive and can readily lend itself to out-patient or community settings.

The Reflective Index (RI) is a parameter derived from the analysis of the DVP., Endothelium-dependent vessel function can be determined by calculating the relative change in reflective index (Δ RI%) following the administration of a Nitric Oxide(NO) releasing $beta_2$-receptor stimulant agent such as Salbutamol. For comparison, endothelium independent vessel function can be similarly calculated by administration of exogenous NO, in the form glyceryl trinitrate (GTN) (Chowienczyk, et al., 1999 ; Kelly, et al., 1990).

2.5.6 Calculation of the arterial stiffness (SI) and endothelial function (ΔRI)

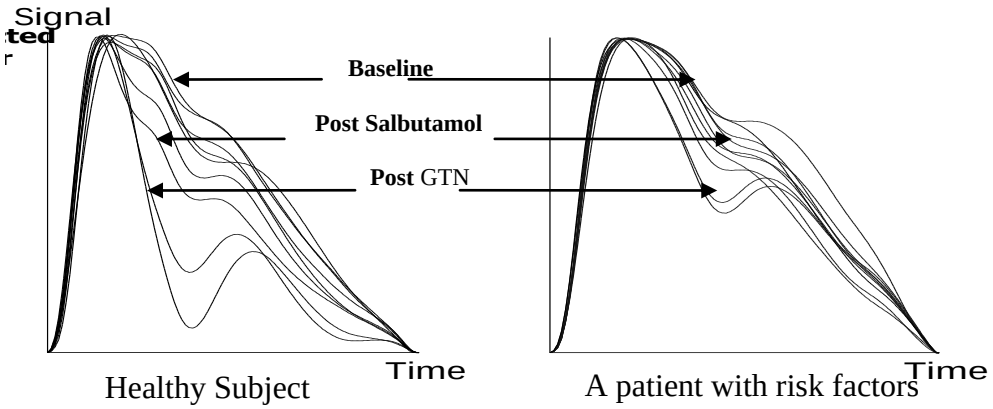
The DVP waveform consists of a systolic peak (a) and a second diastolic peak(b) which is formed by the reflection of the pulse wave from the small arteries in the lower body (Figure 2.1) .The time delay (PPT) between the systolic and diastolic peaks is related to the transit time of pressure waves from the root of the subclavian artery to the apparent site of reflection and back to the subclavian artery. The degree of pulse wave reflection (SI) depends on the impedance of the micro-vascular bed and the tone of the large and small sized blood vessels. This path length can be assumed to be proportional to height (h) and therefore the index of artery stiffness (SI) can be calculated from: SI = h/PPT. The RI is the percentage of systolic pulse wave reflected in each cardiac cycle. Small artery function can be assessed by measuring absolute change in ΔRI from baseline after salbutamol (R_{sal}) and GTN (R_{GTN}) administration. RI can be calculated from: RI=a/b and the endothelial function is determined by: $\Delta RI= (R-(R_{sal})/R)*100$. People with established risk factors are known to have a dysfunctional endothelium and related abnormal wave form response to salbutamol compared to GTN (Figure 2.1b)

Figure 2.1a: Derivation of SI and RI by analysing Digital Volume Pulse wave forms



X = Forward wave / Systolic peak, Y= Reflected wave / Diastolic peak, Reflective Index (RI)= (b / a)* 100, Endothelial function = ($RI_{base\ line} - RI_{Salbutamol} / RI_{base\ line}$)*100

Figure 2.1b: Change in pulse wave form following Salbutamol and GTN Comparing Healthy vs. Individual with risk factors



Healthy Subject A patient with risk factors

2.5.7 Arterial stiffness and endothelial measurement protocol

Measurements of arterial stiffness and endothelial function were performed in the morning following an overnight fast (each subject was instructed to refrain from caffeine-containing beverages, alcohol, and smoking in the previous 12 hours) after which the DVP was recorded in the person's right index finger. Subjects were laid supine, resting for at least 20 minutes in a temperature controlled environment (24±1°C) before the measurements were taken. All the volunteers were advised to refrain from talking and sleeping while the measurements were done. Recorded digital pulse wave forms were used (PCA 2, MicroMedical, Kent, UK) to generate indices of vessel reactivity (RI) and arterial stiffness (SI) using a standard validated protocol (Millasseau, et al., 2006). Each person had at least three measurements (recorded for 30 seconds) taken one minute apart and an average was calculated and used for the analysis. Thereafter, subjects were given a predetermined dose of Salbutamol by inhalation (400 micrograms via a spacer device) and sublingual GTN (400 micrograms) each separated by a washout period of 60 minutes. Relative change in RI was monitored at three minute intervals for a total of 15 minutes. Individuals, whose pulse wave recordings could not be

adequately assessed or who had SI and RI variation greater than 15% within measurements were excluded from the final analysis (11.3%). All of the measurements were performed by the same operator. Intra-observer variation (co-efficient of variation) of the repeated measurements of SI in the same subject on the same day and six weeks later was 5.4% and 7.4% respectively, confirming the findings from recent studies by our group and others that the DVP analysis technique has been shown to have acceptable repeatability and reproducibility (mean difference (SD)(-0.2(4.9%) (Rambaran, et al., 2008)

2.5.8 Quantitative determination of biochemical parameters

Blood samples were collected after three months from the acute event and were taken following an overnight fast, on the same day of the vascular measurements. Blood samples were drawn from an antecubital vein to blood tubes with 3.8% sodium citrate, EDTA and to plain tubes without any chemicals, mixed by gentle inversion, and stored on melting ice. Blood samples were prepared at the Laboratory for Clinical Biochemistry Research, Sandwell and West Birmingham NHS Trust. Plasma fractions were separated by centrifugation at 4°C for 3000g-minutes within 1 hour of collection . Aliquots were stored at -70°C for batch analysis. One aliquot was treated with a cell lysis buffer before storage.

Biomarkers (IL6, hsCRP, TNF alpha, oxLDL, HO, E-Selectin, vWF, Apo A, Apo Apo B, NO, eNOS, cGMP) were measured using the stored blood samples over a six months duration. hsCRP, Apo AI, Apo B were measured using an auto analyser (Cobas Integra 400, Roche, Switzerland). Measurement of all other bio markers were performed by 2-site enzyme linked immunosorbent assay (Please see 2.4., with commercially available antibodies from R&D systems (UK) and Mercodia (USA). The lower limit of detection for Nitrate (0.54 micromol/L), eNOS(25pg/ml), cGMP(1.14 pmol/mL),

HO(0.78ng/mL), oxLDL(<1 mU/l), IL6(0.16 pg/mL), E-Selectin(0.009 ng/mL), TNF(0.391 pg/mL) and intra and inter assay CV (%) for all the ELISA assays were less than 10%. See below.

All the ELISAs performed are standardised within the University Department of Medicine and Sandwell Medical Research Unit Laboratories, Sandwell and West Birmingham NHS Trust, and training was provided by Drs Patel and Blann. Each assay demonstrates intra- and inter-assay coefficients of variation of <10% respectively using the methods detailed in the Appendix & below.

Table 2.2:Intra and inter assay co-efficient variations for measured biomarkers

	Intra assay CV %	Inter assay CV %
Apo A	2.3	3
Apo B	3	4.3
hsCRP	3.4	5.5
oxLDL	7.3	8.3
HO	<10	<10
NO	7.7	2.4
cGMP	7.7	5.6
eNOS	7.4	4.9
IL6	5.8	9.1
eSelectin	6.6	8.7
TNF alpha	7	8.3
vWF	6.1	8.9

2.5.8.1 Measurement of Nitric Oxide level by ELISA technique

The transient and volatile nature of NO makes it unsuitable for most convenient detection methods. However, since most of the NO is oxidized to nitrite ($NO2^-$) and nitrate ($NO3^{--}$), the concentrations of these anions have been used as a quantitative measure of NO production (Moncada, et al., 1989). After the conversion of $NO3^-$ to $NO2^-$, the spectrophotometer measurement of $NO2^-$ is accomplished by using the Griess Reaction (Schreck, et al., 1991). This assay determines nitric oxide based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess

reaction is based on the two-step diazotization reaction in which acidified $NO2^-$ produces a nitrosating agent which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to form the chromophoric azo-derivative which absorbs light at 540 nm (Miles, et al., 1996). The concentration of NO is indirectly measured by determining both nitrate and nitrite levels in the sample.

2.5.8.2 Particle enhanced turbidimetric assay

High sensitivity C-Reactive Protein, Apo A and Apo B were analysed using the Cobas Integra 400 (Roche, Switzerland) auto analyser. The patient sample (serum) was mixed with a reagent containing a suspension of polystyrene latex particles of uniform size coated with rabbit anti-human antibodies (PrecinormProtein). High sensitivity C - reactive protein, Apo A and Apo B in the sample causes aggregation of the polystyrene particles, which is detected and quantified by turbidimetry at 552 nm. Controls (Cat. No. 20766321/ 348 /624) were used as control material with each run. The manufacturer's total coefficient of variation is <10% (see above table).

2.6 Statistics & Validation

2.6.1 Power calculation

In view of the paucity of data concerning all the indices to be measured with regard to South Asian ethnicity, formal power calculation was not possible. Based on previous work by Kalra et al. (2005) and our group, at least 58 people are needed for SI, in order to detect a 0.5SD difference across groups with 80% power and α of 0.05. Therefore it was required to recruit at least 232 subjects, comprising 116 patients with stroke (58 South Asians, 58 European Caucasians) and 116 controls (58 Healthy and 58 with risk factors).

2.6.2 General Statistical methods

Data were first tested for normality using the Kolmogorov Smirnov test. All the normally distributed indices were presented as the mean ± SD, and students' t-test and one-way ANOVA and post-hoc Turkey tests were used to determine differences between groups with continuous variables, and the Chi-square test was used to compare the categorical variables. Central tendencies or medians (IQR, interquartile range) were used to present non parametric data and Kruskal Wallis and Mann Whitney U tests were used to determine differences between groups. For univariate analysis, Spearman rank correlation coefficient (two-tailed) and Pearson correlation coefficient were used to test the relationship between the measured biomarkers, arterial stiffness and other cardiovascular risk indices. Linear regression models were developed for multivariate analysis. All the variables, which were thought to be associated with the dependent variable, were included in the univariate analysis and the variables, which were significant, were subsequently included in the multivariate analyses. Receiver operator characteristics curves was used to evaluate the performance the biomarkers, indices of arterial stiffness and endothelial function indices depicted by the mean area under the curve with 95% confidence interval. A two-tailed p value < 0.05 was considered statistically significant for all comparisons. The range of variation around a mean for a particular continuous variable is calculated using 95% confidence intervals (95% CI). Data were analysed using SPSS versions v14 or v15 (SPSS Inc. Chicago, IL). (Please see individual Chapter methods sections for specific statistical details).

2.6.3 Validation study: Repeatability & Reproducibility of Digital Volume Pulse analysis technique & clinical utility in cardiovascular disease risk stratification

The objective of this validation study was to determine the repeatability & reproducibility of non invasive measurement of arterial stiffness and endothelial function by using DVP analysis technique & assess its clinical utility in cardiovascular disease risk stratification.

2.6.3.1 Introduction

Cardiovascular disease (CVD) is the most common cause of morbidity and premature mortality in the western world (Wild and McKeigue, 1997) (Chapter 1) and has rapidly become an epidemic in the developing world over recent years (Joshi, et al., 2007). Given the considerable healthcare burden conferred by this disease, the timely identification of individuals with an increased risk of CVD is an important consideration.

The assessment of CVD risk amongst individuals is usually performed by calculating 'risk scores', such as the Framingham risk prediction score (Anderson, et al, 1991) and, more recently, European Society of Cardiology (ESC) HeartScore (Conroy, et al., 2003). Risk score estimations use a combination of 'established risk factors', including age, gender, systolic blood pressure, total cholesterol and glycaemic status. However, these scores are known to underestimate actual risk within high risk populations, which has led to the quest for novel risk markers for finer and earlier risk stratification (Danesh, et al., 2004).

Hence, there is a need for novel risk markers (or biomarkers) which are capable of directly examining the underlying pathophysiological processes for refining risk stratification and the early identification of younger high risk populations (Dotsenko, et al.,2007). To be clinically useful in the prediction of CVD, novel techniques should be

closely related to the existing prediction methods. In addition they should also provide an additional value to the existing methods in risk assessment.

Central to our current pathophysiological understanding of CVD is closely allied with accelerated atherosclerosis and age-related arteriosclerosis which are known to alter vessel wall characteristics and increase arterial stiffness. Measures of arterial stiffness indices are accepted as independent markers of CVD. (Laurent, et al., 1994; Cruickshank, et al., 2002). The majority of available methods for measuring arterial stiffness have proven to be both technically difficult to perform and time consuming (Laurent, et al., 2006), specifically in terms of their use in risk assessment amongst large populations and in community settings.

The SI derived from the analysis of Digital Volume Pulse (DVP) is a non invasive indirect technique of measuring arterial stiffness peripherally (Millasseau, et al., 2006). Arterial stiffness measured by the DVP analysis method has been proven to be a validated and reproducible technique with minimal intra-observer variation (Chapter 2). The SI has been demonstrated to have a comparable sensitivity and specificity to the pulse wave velocity method in the identification of patients with latent cardiovascular disease (Woodman, et al., 2005). However, its utility in the cardiovascular risk assessment process amongst an apparently healthy population has not been investigated.

We hypothesised that indices of arterial stiffness (SI) measured by DVP technique are strongly associated with ESC-based cardiovascular risk scores in an apparently healthy population and that the SI is a good discriminator of stratification of CVD risk categories amongst the wider population that includes higher risk individuals. To test this hypothesis, we measured the indices of arterial stiffness in a representative population attending a CVD risk assessment clinic over a one year period.

2.6.3.2 Methods

Using a representative sampling approach, volunteers who originally attended to community based cardiovascular screening clinics (Sandwell and West Birmingham Primary Healthcare Trust, West Midlands, UK) and had similar socio-economic backgrounds based on the Townsend deprivation index and who expressed their willingness to participate in the study were recruited in a subsequent visit. The total cohort (aged 30 to 75 years) comprised apparently healthy people without any known established risk factors (Please see Chapter 2.4.2.1 for details) and people with established risk factors.

Using the European Society of Cardiology (ESC) HeartScore (High Risk) calculator, the absolute CVD risk (%) of developing non fatal coronary heart disease, coronary death or stroke over the next 10 years was estimated. This ESC HeartScore algorithm is based on a European population and has been selected as the new standard in European CVD risk prediction and management by the Third joint European societies task force on CVD prevention in clinical practice (Conroy, et al., 2003). The score is based upon the risk factors age, gender, smoking status, systolic blood pressure, and total cholesterol levels. (Conroy, et al., 2003). Please see Chapter 2.5.6 for details of the measurement of arterial stiffness using DVP analysis technique.

2.6.3.3 Statistical analysis

After being tested for normality using the Kolmogorov Smirnov test, arterial stiffness indices measured demonstrated a normal distribution. Data are presented as the mean ± SD. Student's t-test and one-way ANOVA tests were used to determine differences between groups with continuous variables, and the Chi-square test was used to compare the categorical variables. In univariate analysis Pearson's correlation was used to observe the relationship between arterial stiffness and other cardiovascular risk

indices. Linear regression models were used for multivariate analysis. For the correlation analysis of ordinal variables, Polytomous Universal Model (PLUM) ordinal regression analysis was used, where the Cox and Snell pseudo R Square values are reported to estimate the proportion of the total variation of an ordinal response that is explained by variables included in the model. Receiver operator characteristics curves were used to evaluate the performance of SI, depicted by the mean area under the curve with 95% confidence interval. A two-tailed p value < 0.05 was considered statistically significant for all comparisons. Data are reported to three significant figures. (Please see Chapter 2.6.2 for details).

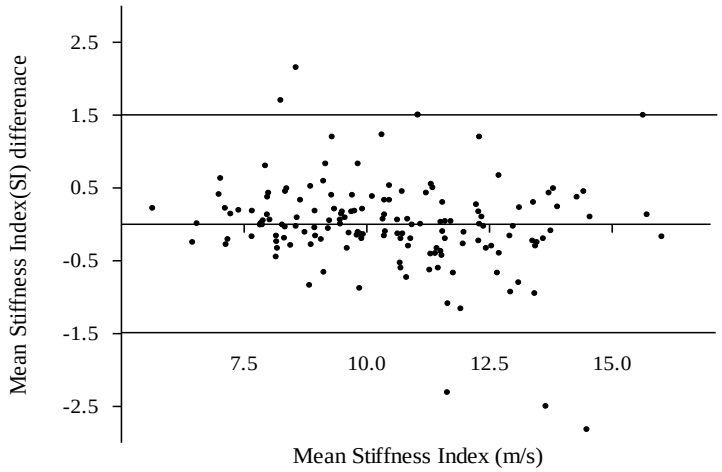
2.6.3.4 Results

2.6.3.4.1 Repeatability and reproducibility of SI measurements

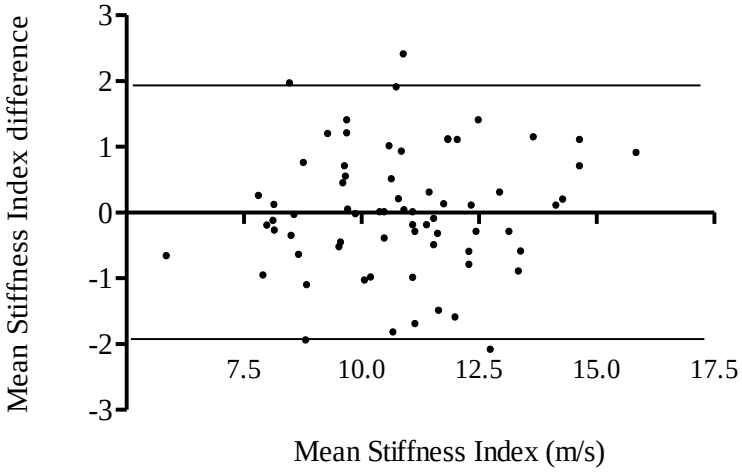
SI was measured a total of three times in each arm at five minutes intervals apart in 164 individuals at the same clinic visit. These measurements were repeated in a separate clinic visit in 64 individuals, in the same temperature controlled environment in six weeks time. Repeated measurements on the same visit, mean difference (SD):0.09(0.66)) as well as in the separate visit (0.12(0.93)) demonstrated a good agreement (Figure 2.2).

Figure 2.2: Bland-Altman test demonstrating the agreement between repeated measurements

(a) Single visit



(b) Repeated visits



2.6.3.4.2 Baseline characteristics:

A total of 187 volunteers including 60 people with risk factors were recruited for this validation sub study (Table 2.3). 57.7% were male. As expected, patients with established CVD risk factors were older, had higher systolic blood pressure and CVD risk scores (P <0.001; Table 2.4). Of the patients with established CVD risk factors, 33% had diabetes mellitus, 77.8% had a diagnosis of hypertension and 61% had hypercholesterolemia. Of these patients, 38.9% were on regular antihypertensive

medications, 11.1% were on anti-diabetes medications, 11.2% were on anti-cholesterol treatment and 14.4% were on anti-platelet treatment

SI was significantly higher in males (P=0.01), as well in smokers (P=0.006), those with a history of hypertension (P=0.007), diabetes (p=0.02), hypercholesterolemia (P=0.002) and those with a high waist hip ratio (P=0.001), but not with those with a higher body mass index (P=0.49). Measurements of SI was also significantly higher in people with established risk CVD factors without any gender variation (P <0.01).

2.6.3.4.3 Relationship to ESC based CVD risk stratification

Patients without complete CVD risk score data (17.6%) were excluded from the risk stratification analysis. On univariate analysis there was a positive association between SI and CVD risk (Pearson correlation coefficient (r) : 0.56, P=<0.001). On linear regression analysis SI was associated with CVD risk scores (Beta (SD):0.58(1.3-2.0); P<0.001). SI increased in an ordinal fashion across from low risk (<5%), medium (5-10%) high (11-19%) and highest risk (>20%) (Pseudo R-Square = 0.30; P<0.001) (Figure 2.3). Of the total population, 48.7% had lower-medium CVD risk (<15%) and correspondingly lower SI values (mean ± SD: 7.9(1.5) ms^{-1} (Figure 2.3) compared to those at high risk (P<0.001). In addition, male subjects had a higher CVD risk scores (P<0.001) and correspondingly higher SI compared to the females (P=0.01). There was a significant difference in the mean risk score levels (P<0.001) and SI between healthy volunteers and risk factor controls in both males (P=0.008) and females (P=0.015) (Table: 2.3).

Table 2.3: Baseline characteristics comparing healthy vs. people with risk factors

Cardiovascular risk factors Mean(SD)	Male (n=108)			Female (n=79)		
	Healthy volunteers (n=76)	Risk controls (n=32)	p Value*	Healthy volunteers (n=51)	Risk controls (n=28)	p Value*
Age(years)	45.6(13.2)	65.8(10.4)	<0.001	45.9(12.5)	63.6(10)	<0.001
Height (m)	1.69(0.1)	1.69(0.2)	0.97	1.59(0.09)	1.58(0.05)	0.12
Body mass index(Kg/m^2)	25.5(3.4)	27.9(3.2)	0.02	26.5(3.1)	26.9(4.4)	0.49
Waist hip ratio	1.01(0.1)	0.99(0.1)	0.27	0.98(0.1)	0.93(0.1)	0.28
Mean systolic BP(mmHg)	137(17)	145(15)	<0.001	126(16)	150(15)	<0.001
Mean diastolic BP(mmHg)	84(11)	82(13)	0.38	78.(10)	83(10)	0.06
Mean arterial BP(mmHg)	101(12)	103(13)	0.04	94(11)	106(10)	<0.001
Serum Cholesterol (mmol/l)	4.52(0.85)	4.1(0.4)	0.08	4.49(0.89)	5.1(1.1)	0.01
Fasting plasma glucose(mmol/l)	4.04(1.59)	3.9(1.2)	0.87	3.55(1.4)	3.3(1.5)	0.67
Smoking (%)	8.2	11.1	0.44	6.5	8.3	0.45
Alcohol (%)	9.8	15.6	0.42	8.2	12.8	0.41
ESC CVD risk score(%)	7.4(6.1)	13.1(5.9)	<0.001	4.58(4.6)	11.3(4.7)	<0.001
Stiffness index(ms^{-1})	9.3(2.2)	10.9(2.4)	0.008	8.4(2.1)	10.1(2.4)	0.015

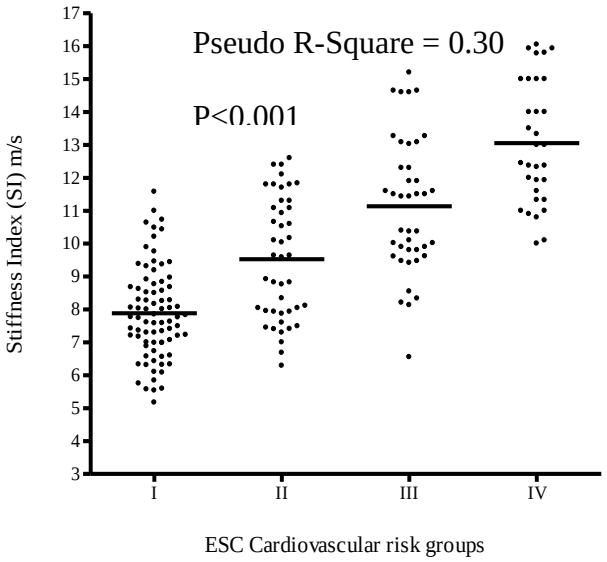
ESC: European Society of Cardiology, CVD: Cardiovascular disease, BP: Blood Pressure, *p value comparing healthy vs. risk controls; significance at 0.05 level, Mean (SD)

Table 2.4: Indices of arterial stiffness for the total cohort

Group characteristics		% of all patients (n-187)	Mean arterial stiffness ms^{-1} (SD)	p value*
Gender	Male	57.7	9.6(2.3)	0.01**
	Female	42.3	8.8(2.2)	
Waist hip ratio	<0.9	40.1	7.9(1.6)	0.001
	>1	59.9	9.4(2.3)	
Body mass index (kgm^{-2})	I(<25)	33.5	9.3(2.5)	0.85
	II(>25)	66.5	9.5(2.2)	
Smoking	Yes	10.2	10.6(2.5)	0.006**
	No	89.8	9.1(2.2)	
Alcohol	Yes	9.6	10.1(2.3)	0.5**
	No	90.4	9.6(1.5)	
Diabetes	Yes	5.6	11.2(2.4)	0.02**
	No	94.4	9.9(2.3)	
Hypertension	Yes	18.3	10.4(2.2)	0.007**
	No	81.7	9.1(2.5)	
Hypercholesterolemia	Yes	13.3	10.8(2.8)	0.002**
	No	86.7	9.0(2.2)	

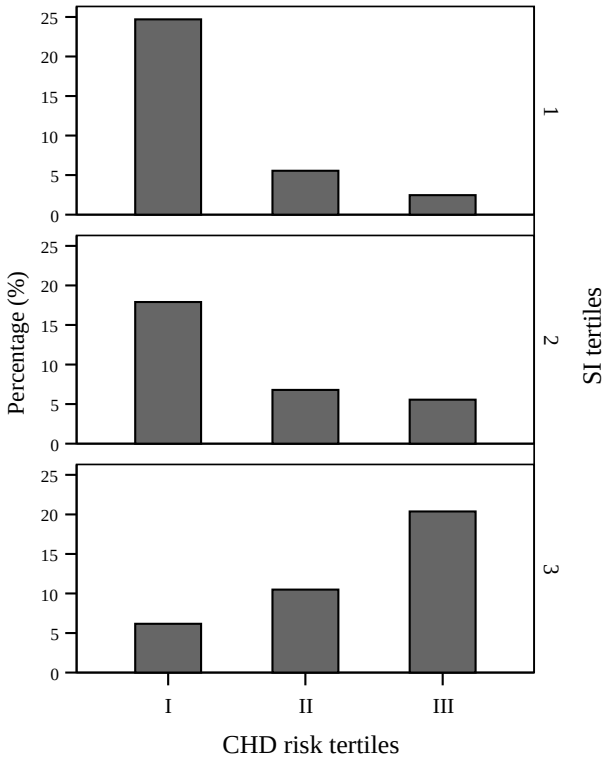
*p value using one way ANOVA across all groups and independent t-test; significance at 0.05 level. SD: Standard Deviation. ** p value using chi square

Figure 2.3: Ordinal association between Stiffness Index and ESC risk score groups



European Society of Cardiology (ESC) HeartScore based cardiovascular disease (CVD) risk groups(%): Group I (<5%), Group II (6-15%), Group III (16-19%), Group IV(>20%). Line shows the mean CVD risk score

Figure 2.4: Distribution of SI and CVD risk groups according to tertiles



European Society of Cardiology (ESC) HeartScore based Cardiovascular disease (CVD) risk tertiles (%): Tertile I= (<5%), Tertile II =(6-15%), Tertile III= (> 16%). Stiffness Index (SI) tertiles (ms^{-1}): Tertile 1 = SI<7.7; Tertile 2= SI 7.8-9.7; Tertile 3= SI>9.8

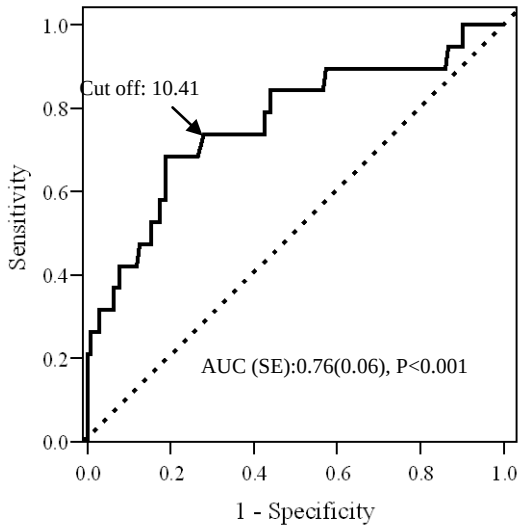
2.6.3.4.4 Correlations and multivariate regression

On univariate analysis (excluding people with established risk factors and medications) there was a significant positive association (r) between SI and Age (r= 0.41; P<0.001) mean systolic (r= 0.24;P<0.001), diastolic blood pressure (r=0.29;P=0.001), pulse pressure (r=0.17;P=0.03) and mean arterial pressure (r=0.34;P=0.003) There was no significant association between SI and mean heart rate, body mass index, waist hip ratio, fasting plasma glucose and serum cholesterol. In multivariate regression analysis, Beta (95% CI) age (0.11(0.04-0.17); P< 0.002), waist hip ratio (8.8(2.3-17.8); P=0.05) and mean arterial pressure (0.06(0.01-0.11); P=0.01) independently associated with SI but not with serum cholesterol, fasting plasma glucose levels or heart rate.

2.6.3.4.5 Receiver Operator Characteristic (ROC) curve analysis

ROC analysis of baseline characteristics to discriminate subjects with higher CVD risk, found SI to be the most useful variable in this population (Area Under Curve (AUC)(SE) :0.76(0.06), P<0.001) (Figure 2.5) compared to, total cholesterol (AUC:0.58(0.07), P=0.17) plasma glucose (AUC :0.62(0.07), P=0.07), mean blood pressure (AUC:0.60 (0.06), P=0.15) and waist hip ratio (AUC:0.45(0.04), P=0.35). In addition, SI had the discriminatory utility to identify the patients with known diabetes (AUC 0.68 (0.04), P<0.001), hypercholesterolemia (AUC 0.66 (0.03), P<0.001) hypertension (AUC 0.66 (0.03), P<0.001), and higher waist-hip ratio (AUC 0.69 (0.05), P=0.001) but not with higher body mass index (AUC 0.50 (0.06), P=0.92).

Figure 2.5: ROC analysis of SI in discriminating high CVD risk group



AUC: Area Under the Curve, SE: Standard Error

2.6.3.5 Discussion

In this study we demonstrate the repeatability and reproducibility of DVP analysis technique to measure arterial stiffness and its clinical utility to stratify CVD risk. The current study demonstrates a close association between SI and CVD risk score estimation using the ESC HeartScore model. More importantly, the discriminatory properties of the SI in identifying higher risk groups were significantly better than those of conventional cardiovascular risk indices, such as total cholesterol level and fasting blood sugar measurements.

Screening of the population with ESC HeartScore and Framingham-based risk score methods continue to be recommended in many current guidelines (JBS 2, 2005). However the absolute levels of cardiovascular risk factors are mathematically combined as a holistic approach during risk prediction, with limited direct relationships to underlying pathophysiological changes, as in our current analysis known to underestimate around 16%-20% of the CVD risk (Danesh, et al., 2004). This suggests that a significant proportion of the apparently healthy but 'higher risk' subjects may be

missed in a traditional risk assessment process and highlights the importance of using potential new risk markers to aid more conventional cardiovascular risk stratification schemes (Sutton-Tyrrell et al., 2005).

The novel DVP technique used to assess the arterial distensibility of volunteers in this study is an easily performable, non-invasive technique with low intra-observer and inter-observer variation. This method allows the indirect examination of the structural integrity of both large and small arteries simultaneously (Millasseau, et al., 2006) (allowing the identification of apparently healthy individuals with sub-clinical atherogenesis and premature arteriosclerosis. Measurements of SI may therefore be more useful in the early identification of high risk subjects without established risk indices such as high blood pressure or cholesterol levels. Such people are usually omitted in traditional CVD risk assessments.

As expected, subjects with established risk factors in our study also demonstrate higher SI when compared to healthy controls. Moreover, the SI was a better discriminator in identifying people with established CVD risk factors (such as hypertension, diabetes and hypercholesterolemia) where individual point measurements of theses risk indices are less useful, for example, where they are taking medications. Whilst the present study highlights the clinical utility and acceptability of DVP measurements in a wide spectrum of individuals with and without established risk factors, further studies are warranted to assess the utility of SI in clinical practice and to monitor treatment efficacy as well as disease progression.

In our study, the principle factors contributing to increased SI include age and mean arterial pressure but not body mass index. This is in keeping with the other published studies in which the pulse wave technique was used (Westerbacka, et al., 2001). The independent association between waist to hip ratio with increased SI merits

careful consideration. Indeed, several studies have reported an association between waist to hip ratio and cardiovascular risk factors such as hypertension, and lipid and glucose concentrations (Pouliet, et al., 1994). People with a raised waist to hip ratio are also known to have varying degrees of insulin resistance (Westerbacka, et al.,2001) and have been shown to increase in patients with obesity (Czernichow, et al., 2005). The systemic effects of insulin resistance, such as increased adrenergic response, sympathetic tone and vascular inflammation are some of other mechanisms that may be involved in increased arterial stiffness.

Limitations:

The limitations of the current study include the cross-sectional nature of the design and use of indirect indices to measure arterial stiffness. However other invasive complex measurements of arterial stiffness are not practical to use as a tool to screen larger populations in the clinical settings. In the current analysis, other metabolic, inflammatory biomarkers and the impact novel risk indices on arterial stiffness in this population have not been examined. Diagnosis of diabetes mellitus was also made on the basis of fasting blood glucose measurements and available documented evidence. None of the volunteers had an oral glucose tolerance test for specific disease exclusion. In the current study CVD risk calculation was based on a European risk score (ESC based *Heart Score*), which does not use HDL cholesterol or diabetes status for risk estimation compared to the Framingham (US) based risk calculation which allow finer risk stratification of higher CVD risk individuals with lower HDL values and diabetes.

In addition, the DVP method used to measure arterial stiffness in the current study did not provide information on individual contributions that both large and small arteries make towards wave reflection and overall arterial stiffness. Further calculation of SI is based on the assumption that the subject's height is proportional to the path length

of the wave reflection. In addition this study may not have the power to discriminate or compare the established risk factors such as smoking status between healthy and risk factor control groups. Moreover this study does not provide the facility to determine the discriminatory utility of SI in different age categories. Using a larger prospective study design which used CVD outcomes with combined methods of measurement (Pulse Wave Velocity and DVP, for example) would possibly have provided more comprehensive details thereby giving greater explanatory power to this study. However, the logistics for such a study are also limited by the need to treat individuals recognized to be at increased CVD risk.

In conclusion, indices of arterial stiffness (SI) measured using the DVP technique is strongly associated with the ESC 'HeartScore' cardiovascular risk score and demonstrates the discriminatory utility of the Stiffness index in identifying high risk populations. Thus, non-invasive measurement of arterial stiffness may aid the identification of individuals with high cardiovascular risk. However there is a need for future external validity studies of stiffness index to demonstrate the ability to prospectively predict the clinical outcomes over and above those predicted by existing CVD risk score estimations.[1]

[1] Gunarathne, A., Patel, J.V., Hughes, E.A., et al. (2008).Measurement of stiffness index by digital volume pulse analysis technique:. **American Journal of Hypertension**,21:866-72

Chapter 3 : The differences in prevalence of cardiovascular risk profile and mortality amongst South Asian stroke survivors

Literature appraisal (Chapter 1) indicates a significant paucity of comparative cardiovascular risk profile data in South Asian stroke survivors. Therefore, as a step towards understanding the pathophysiology of cerebrovascular disease amongst this higher risk population, this chapter intends to examine the differences in prevalence of cardiovascular risk profile and related stroke mortality in a larger cohort of stroke population.

3.1 Introduction

Stroke is a leading cause of premature and preventable death in the UK, and accounts for more than 10% of all deaths globally (Tolonen, et al., 2002). Together with its well known morbid affliction in survivors, it is a key focus for national healthcare and research (Lip, et al., 2007 and Rothwell et al., 2005). The burden of ischaemic stroke is more prominent amongst the country's migrant communities (Wild and McKeigue, 1997) and this relates to the impact of its determinants amongst these ethnic minorities, most notably, hypertension and diabetes mellitus. (Raleigh, et al.,1997; Khattar, et al., 2000; Cappuccino, et al.,2003; Schulz and Rothwell, 2003; Lane, et al., 2005; Lane and Lip 2005). Stroke mortality is markedly greater amongst South Asians living in Britain, where both men and women have a 40% higher risk of mortality after stroke than indigenous European Caucasians (Wild and McKeigue, 1997). Whilst rates of mortality from stroke continue to decline in the UK, the rate of regression is markedly less in South Asians (Gill et al., 2007).

Previous epidemiological analyses in the United States have been a valuable insight into the relationship between ethnic variations in ischaemic stroke mortality and

the disproportionate distribution of cardiovascular (CVD) risk factors in these groups (Gillum, et al., 1995 and Gillum, et al., 1999).

Migrant communities living in the UK have experienced large lifestyle changes, the effects of which are likely to explain the insidious CVD risk profiles manifest in these ethnic groups (Cruickshank and Alleyne, 1987; Bhatnagar, et al., 1995; Patel, et al., 2006). Historically, some studies amongst migrant groups in Britain also suggested that some CVD risk factors were absent in these groups, specifically hyperlipidaemia (Cruickshank, et al., 1991). However, there is limited information on how trends in CVD risk factors and stroke in South Asians have changed in recent years, particularly so in the current decade.

Previous studies in the US have identified aetiological and survival differences in stroke amongst principal ethnic groups (Friday, et al., 1989). However, longitudinal data demonstrating the association between individual cardiovascular (CVD) risk factors and stroke mortality in South Asians in the UK are not available Specifically, the increased CVD risk in South Asians populations is thought to be related to an innate susceptibility to diabetes mellitus (Patel, et al., 2005). However, the impact of diabetes on long term stroke mortality in South Asian is unknown.

The objective of the present study was to investigate ethnic differences in the CVD risk profile and mortality of stroke admissions to an inner city teaching hospital serving a multi ethnic population in Birmingham, United Kingdom. We tested our null hypothesis that there would be no significant inter-ethnic differences in the CVD risk profile and 30-day all cause mortality of stroke admissions, nor any secular trends that were evident over a nine year period (1997-2005). Further, we hypothesised that stroke mortality in South Asians was independently related to diabetes, and the strength of this association is greater than that for other established CVD risk factors.

3.2 Methods

3.2.1 Study design: Retrospective analysis of stroke registry data

A stroke registry was developed using patients' information system and electronic case notes to determine prevalence of cardiovascular risk profile in a multi ethnic population admitted to Sandwell and West Birmingham NHS Trust over a nine year period (1997-2005). All the patients who initially admitted with a "first-in-lifetime" ischaemic stroke to Sandwell and West Birmingham NHS Trust between 1997-2005 period (International Classification of Disease (ICD) 10^{th} revision, ICD code: 163) were included in the study. Please see Chapter 2.4.1 for details on development of the stroke registry, diagnostic criteria and definitions.

Patients who were identified as South Asians (Indian, Pakistani, Sri Lankan, Nepalese, and Bangladeshi (self-reported ethnicity)) were included in the five year mortality analysis. Data from the stroke registry was combined with five years follow up data (1997-2001) obtained from the national mortality tracing system to determine events-free survival rates amongst South Asians admitted to our Sandwell and West Birmingham NHS Trust over a five year period.

3.2.2 Data Analysis

Data were analysed in SPSS version v14 (SPSS Inc. Chicago, IL). (Please see chapter 2.6 for details). The hospital admission rate within a time-period was calculated using the total number of hospital admissions for that period, and the stroke admissions for that time as the numerator. Student's t test and one-way ANOVA tests were used to determine differences between groups with continuous variables and Chi-square test was used to compare the categorical variables (e.g. differences in the prevalence of risk factors between secular time points). The prevalence of risk factors were age adjusted using the age distribution of total hospital admissions (1997–2005). 95% confidence

intervals (CI) were calculated for proportions (P) using the formula, CI = √ (P * (1-P) / n).

Kaplan-Meier plots were used for univariate survival analysis. In Multivariate analysis, adjusting for patient's (age and gender) stepwise Cox regression models were developed to identify independent predictors of mortality. For the longitudinal analysis, Hazard Ratios (HR) and their 95% confidence intervals (CI) for death were estimated. To illustrate the strength of a predictor of mortality, beta (95%CI) values were calculated. Statistical significance was accepted at the 0.05 level.

3.3 Results

Between 1997-2005, there were 3083 hospital admissions which were first-in-lifetime-strokes, where complete clinical data and mortality data were available. Of these patients, 2405 (78.0%) presented with a non-haemorrhagic stroke, 159 (5.2%) with a haemorrhagic stroke and 519 (16.8%) were unspecified. Of all strokes, 46.6% were males, who were significantly younger than female patients (mean (SD) 72.1 (10.6) years vs. females, 77.1(10.9) years; P < 0.001). South Asians were younger at admission compared to European Caucasians. (P < 0.001) (Table 3.1). Afro-Caribbeans were excluded from the comparative analysis (11.4%).

3.3.1 Risk factors

Amongst those patients admitted with non-haemorrhagic stroke, hypertension was the most commonly encountered cardiovascular co morbidity (62.6%) followed by diabetes mellitus (35.0%) and atrial fibrillation (27.4%). South Asians had a higher prevalence of diabetes and hypertension compared to European Caucasians (P<0.001), whereas atrial fibrillation and heart failure were more common in the European Caucasian population (P< 0.001). South Asians also had a higher prevalence of

myocardial infarction and hyperlipidaemia compared to European Caucasians. (P<0.05) (Table 3.2).

3.3.2 Secular trends in non haemorrhagic stroke admissions

Between 1997-2005 there was a secular decline in admission rates of non-haemorrhagic stroke (P<0.001) from 1.6 per 1000 admissions in the period 1997-1999 to 0.95 in the period 2003-2005. Between ethnic groups, the magnitude of the secular decline in rates of non-haemorrhagic stroke admissions varied, where the decrease amongst European Caucasian was greater than those observed in the South Asian population (P<0.03).

3.3.3 Secular trends in cardiovascular risk factors

Hypertension, peripheral vascular disease and hyperlipidaemia were all increased between secular time periods between 1997-2005 ($p < 0.001$). Myocardial infarction was the only risk factor which showed a significant decrease in prevalence during the same periods for the total stroke population ($p<0.001$). Amongst the South Asians, hypertension ($p = 0.026$), peripheral vascular disease ($p = 0.019$) and hyperlipidaemia ($p < 0.001$) showed a significant increase during the period 1997-2005. During the same period, European Caucasians also showed a significant increase in the prevalence of hypertension ($p < 0.001$), hyperlipidaemia ($p < 0.001$) and peripheral vascular disease ($p = 0.003$). In contrast, myocardial infarction was decreased in the European Caucasian population ($p<0.001$). (Figure 3.1, Table 3.1).

3.3.4 Duration of stay and in-hospital all cause mortality

The average duration of hospital stay for the total population was 20.9 (SD ±5.2) days. Duration of hospital stay was comparable between European Caucasians and South Asians (20.9 and 21.63 days). On survival analysis, cumulative event-free survival (all cause mortality) over 30 days varied significantly by ethnicity, but significantly

decreased in all groups (P=0.031). European Caucasians had the poorest survival between1997-1999 (P= 0.02), while South Asians had the poorest survival for the time periods 2000-2002 (P= 0.29) and 2003-2005 (P= 0.03) (Table 3.1).

On Cox regression analysis of 30 day all cause mortality, incorporating age, gender, ethnicity and cardiovascular risk factors (hypertension (odds ratio, 95%CI) 1.51 (1.15-1.97), P = 0.028) and age (1.02 (1.01-1.03), P = 0.001) were independent predictors in the first time period. Between 2000-2002, hyperlipidaemia was a non-significant predictor of 30 day mortality (P = 0.06). Between 2003-2005, South Asian ethnicity was an independent predictor of mortality vs. European Caucasians (2.47 (1.10- 5.54), P = 0.03).

Table 3.1: Secular trends in demographics and Cardiovascular risk profile 1997-2005

	1997-1999 (n=986)	2000-2002 (n=690)	2003-2005 (n=729)	P Value
Mean Age	73.9(10.9)	74.8 (11.0)	75.8 (11.1)	< 0.01
Male	467 (47.4%)	325 (47.1%)	329 (45.1%)	0.02
Female	519 (52.6%)	365 (52.9%)	400 (54.9%)	0.53
Ethnic Group Breakdown n (%)				
Caucasian Male	319 (32.3)	194 (28.1)	218 (29.9)	< 0.001
Caucasian Female	403 (40.9)	264 (38.3)	311 (42.7)	< 0.001
South Asian Male	85 (8.6)	83 (12.0)	75 (10.2)	0.71
South Asian Female	60 (6.1)	60 (8.9)	57 (7.8)	0.95
Hospital Admission Rates n(CI)				
Total	1.60 (1.5-1.7)	1.06(1.0-1.1)	0.95 (0.9-1.0)	<0.001
Caucasian	1.60 (1.5-1.7)	1.06(1.0-1.1)	0.99(0.9-1.0)	<0.001
South Asian	1.19 (1.1-1.3)	0.98 (0.9-1.1)	0.86(0.8-0.9)	0.03
Risk Factors n (%)				
Hypertension	539 (54.7)	446 (64.6)	515 (70.6)	< 0.001
Myocardial infarction	93 (9.4)	38 (5.5)	26 (3.6)	< 0.001
Peripheral vascular disease	50 (5.0)	31 (4.5)	71 (9.7)	< 0.001
Hyperlipidaemia	33 (3.3)	61 (8.8)	112 (15.4)	< 0.001
Atrial fibrillation	267 (27.1)	177 (25.7)	211 (28.9)	0.38
Diabetes	367 (37.2)	249 (36.1)	269 (36.9)	0.89
Ischaemic heart disease	228 (23.1)	156 (22.6)	185 (25.3)	0.41
Valvular heart Disease	23 (2.3)	14 (2.0)	15 (2.1)	0.89
Transient ischaemic attacks	72 (5.7)	73 (5.8)	96 (7.7)	0.15
Duration of stay (days) (SD)	24.8 (1.6)	20.7 (1.6)	17.9 (1.5)	< 0.001
Stroke Mortality Rate (CI)	319 (275-364)	303 (257-349)	273 (226-320)	0.03

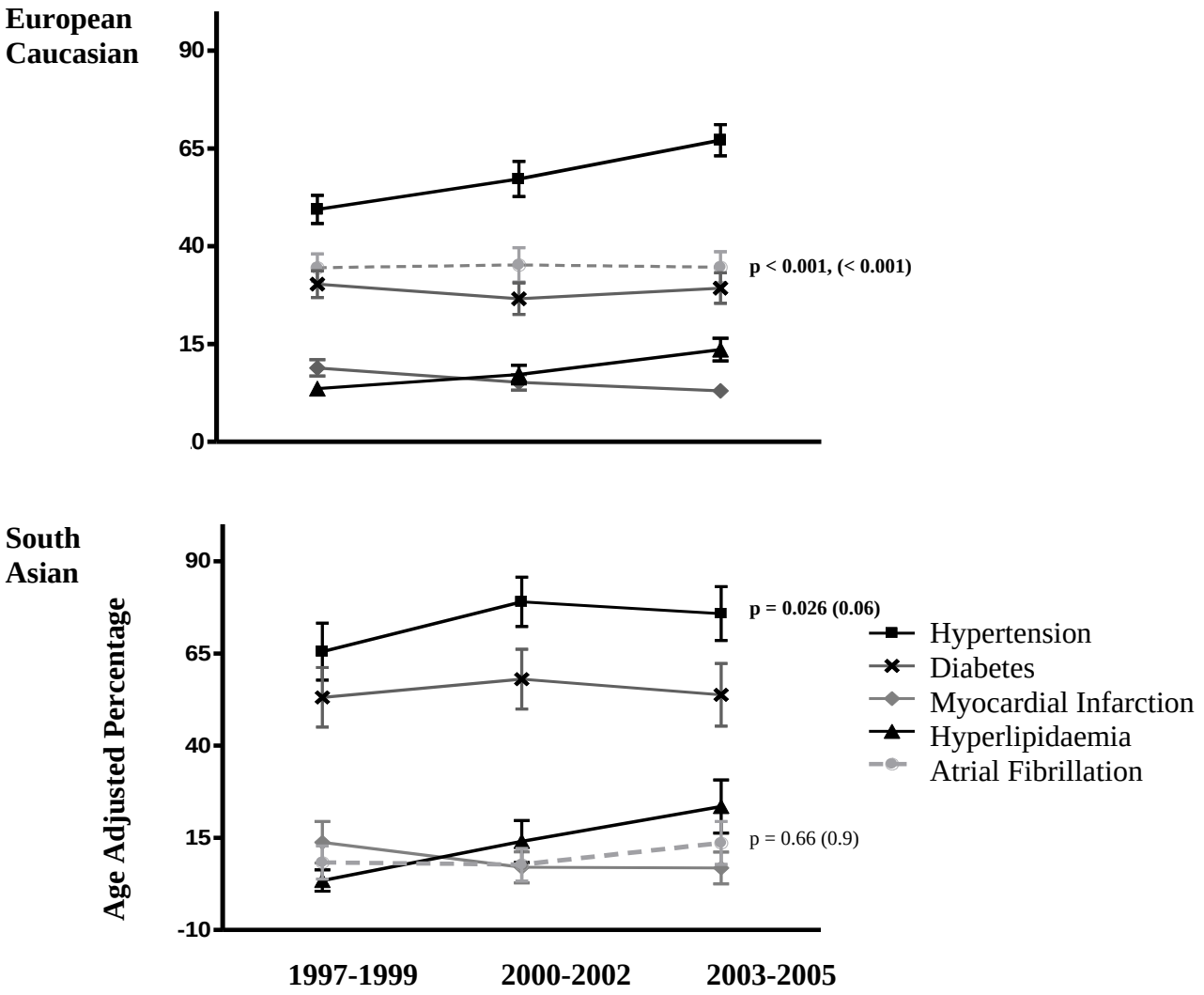
(P value using chi square analysis, except age of admission where ANOVA is used. Data are n (%) or mean (SD) and rates are per 1000 patients per year (95%CI)).

Table 3.2: Cardiovascular risk profile by ethnic group 1997-2005

	Total sample (n=2405)			**South Asian (n = 420)**			**European Caucasian (n=1709)**			**p value***
Age years (SD)	T	M	F	T	M	F	T	M	F	
	74.7 (11.0)	72.1 (10.6)	77.1 (10.9)	68.8 (11.4)	68.3 (11.3)	69.4 (11.6)	76.7 (10.5)	73.4 (10.3)	79.2 (9.9)	<0.001**
Male: Female ratio		0.87			1.37			0.75		
Risk Factors n (%)										
Hypertension	62.6 (60.7-64.5)			73.7 (69.5-77.8)			58.6 (56.3-60.9)			< 0.001
Diabetes	35.0 (33.2-36.9)			50.3 (45.6-55.0)			29.8(27.6-31.9)			< 0.001
Atrial fibrillation	27.4 (25.6-29.1)			11.8 (8.7-14.8)			34.8 (31.9-36.8)			< 0.001
Heart failure	11.6 (10.3-12.9)			7.0 (4.6-9.4)			13.0 (11.4-14.5)			< 0.001
Hyperlipidaemia	9.2 (8.0-10.3)			10.8 (7.9-13.7)			9.2 (7.8-10.5)			< 0.01
Myocardial Infarction	6.4 (5.4-7.3)			9.1 (6.4-11.8)			6.2 (5.1-7.3)			0.03

Data are mean (SD) or age adjusted percent (95%CI) * based on Chi square test across all groups. T; total, M;male, F;female

Figure 3.1: Secular trends in the CVD risk profile 1997-2005 by ethnicity



P values using chi square analysis comparing 1997-1999 period vs. 2003-2005 period. Error bars show 95% confidence intervals. * p value for Hyperlipidaemia. ** p value for Atrial Fibrillation

3.3.5 Five year all cause mortality analysis in South Asian stroke patients

Between 1997 and 2001, there were 1474 first in lifetime stroke admissions, of which 340 (23%) were in South Asian individuals. Complete clinical data and five year follow up mortality data were available for 326 patients. Of South Asian patients, 242 (74.3%) had ischaemic strokes, 8.6% had haemorrhagic strokes, 1.9% had sub-arachnoid haemorrhages and 15.3% were unspecified. Hypertension was the most prevalent risk factor, being present in 70.2%, followed by diabetes mellitus in 56.2%. AF was recorded in 7.4 % of the patients, whilst 10.3% had a history of MI. In 21% of the patients, diabetes was the only comorbidity present (Table 3.3). In total, 46% of patients had more than one risk factor, of which diabetes and hypertension was the most common risk factor combination. There were no gender differences in the distribution of CVD risk factors.

At 5 years follow up of the patients who had an ischaemic stroke, 99 subjects (40.5%) had died. The median duration of survival for the total population was 150 (IQR 8.75-924) days. There were no gender differences in mortality either cumulatively over 5 years or within each year (log rank test, P=0.49) (Figure 3.2). Cumulative event-free mean survival at 5 years was significantly poorer in patients with diabetes without other risk factors (log rank test, P=0.009) (Figure 3.3). On univariate analysis, Hazard Ratio's for individual risk factors were age:1.01(0.9-1.03); P=0.14, gender:0.86(0.57-1.3);P=0.49, diabetes:1.7(1.14-2.7); P=0.01, hyperlipidaemia:0.63(0.25-1.5); P=0.31, hypertension:0.74(0.47-1.15);P=0.18, myocardial infarction:0.6(0.3-1.1);P=0.17 and heart failure: 0.82(0.36-1.8);P=0.64. On Cox regression analysis, incorporating age, gender and other CVD risk factors, diabetes mellitus was an independent predictor of mortality 1.66(1.02-2.6), P=0.039) (Table 3.4).

Figure 3.2: All cause mortality in South Asian stroke patients in relation to duration of follow up

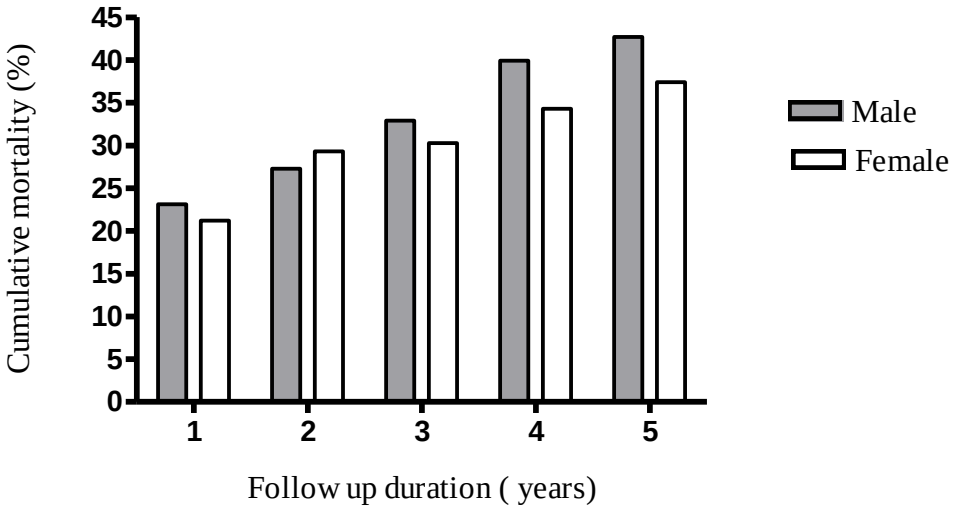


Figure 3.3: Kaplan-Meier plot demonstrating five year event free survival in South Asian stroke patients

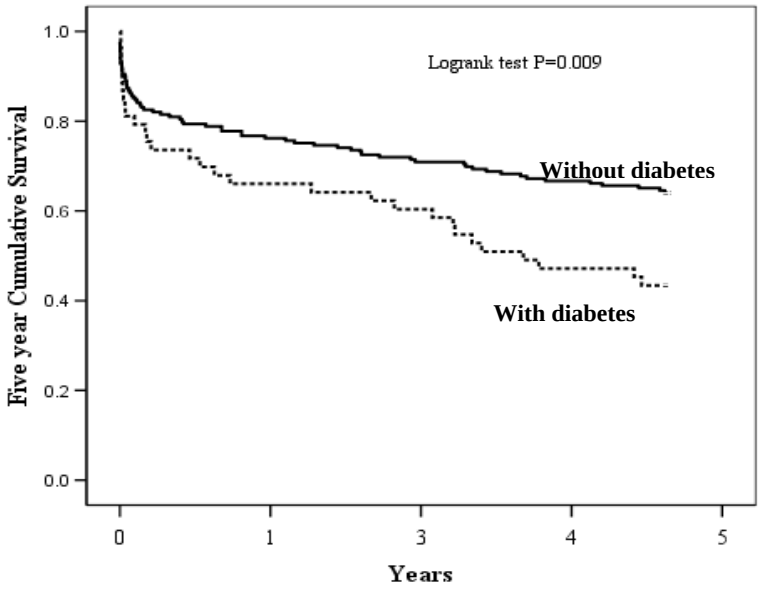


Table 3.3: Cardiovascular risk profile for South Asian stroke patients

	Total sample (n=242)	Male (n = 143)	Female (n=99)	P value*
Age years(SD)	68.0 (10.9)	67.2 (10.2)	69.7 (11.8)	0.13
Cardiovascular risk profile % (95% CI)**				
Hypertension	70.2 (64.4-76.0)	72.7 (65.4-80.0)	66.7 (57.4-76.0)	0.31
Diabetes	56.2 (50.0-62.5)	57.3(49.2-65.4)	54.5(44.7-64.3)	0.66
Atrial fibrillation	7.4 (4.1-10.7)	5.6 (1.8-9.4)	10.1 (4.2-16.0)	0.19
Heart failure	7.4 (4.0-10.8)	7.7 (3.3-12.1)	7.1 (2.0-12.2)	0.86
Hyperlipidaemia	7.0 (3.8-10.2)	7.0 (2.8-11.2)	7.1 (2.1-12.3)	0.98
Myocardial infarction	10.3 (6.5-14.1)	11.9 (6.6-17.2)	8.1 (2.7-13.3)	0.34

Table 3.4: Independent determinants of five year stroke mortality amongst South Asians (Cox regression analysis)

Variables	Hazard Ratio (95% CI)	P value*
Age	1.01 (.99-1.03)	0.12
Atrial fibrillation	0.82 (0.19-3.4)	0.64
Hyperlipidaemia	0.74 (0.3-1.87)	0.54
Hypertension	0.84 (0.52-1.39)	0.51
Diabetes	1.66 (1.02-2.6)	0.04
Myocardial infarction	0.66 (.368-1.19)	0.17
Heart failure	0.88(0.38-2.04)	0.88

3.4 Discussion

Contrary to our null hypothesis, there was a decline in rates of hospital admissions with non-haemorrhagic strokes for the total studied population in recent years. However, the prevalence of hypertension and hyperlipidaemia was seen increased over the studied period, but may be explained by how the identification of risk factors has improved among stroke patients, in line with a holistic approach in CVD risk management, specifically in South Asians (JBS 2, 2005). The data here give an insight into the changing CVD risk profile amongst ethnic groups in this stroke population but may not truly reflect the changes in the general population. In South Asians, the presence

of an adverse CVD risk profile is likely to explain why rates of stroke have not fallen to the same extent as those seen in other ethnic groups and merits further investigations.

In recent years, there has been consensus recognition that South Asians are a high risk group for CVD, and this may have promoted heightened CVD risk assessments specific to this population. Thus, it would be important to investigate the role of dyslipidaemia amongst South Asians in a community-based study, in view of its impact on stroke aetiology and pathophysiology. Using a hospital based approach, South Asian stroke survivors have been found to have significantly higher Apo lipoprotein B to A ratios, and higher Lipoprotein(a) levels when compared with ethnically-matched healthy controls (Sharobeem, et al., 2007).

Given that hyperlipidaemia is a commonly encountered risk factor in CVD, being present in more than 25% of the stroke patients, there is the need to determine the importance of dyslipidaemic management in the treatment and prevention of stroke in a multi-ethnic population. In two other reported South Asian stroke studies, 50% of the stroke patients had elevated cholesterol levels (Deleu, et al., 2006; Anand, et al., 2000) . In European Caucasians, the prevalence of hyperlipidaemia ranges from 28.7 to 32% (Sacco et al 2001). In the present study, the prevalence of hyperlipidaemia increased by fivefold in South Asians over the 1997-2005 period. This raises the possibility of adverse transition of the CVD protective traits in hyperlipidaemia, which were a feature of early migrant populations, to a more atherogenic profile as described in the indigenous population (Bhatnagar, et al., 1995). The majority of the other stroke studies in UK have supported a similar decline in the prevalence of stroke, (Tolonen, et al., 2002) but the pace of decline in stroke prevalence in South Asians was not significant, reaching a nadir across the three time periods. This is consistent with the demonstrated adverse transition of CVD risk profile amongst South Asians shown here.

This study also shows a declining trend in in-hospital all cause mortality rates in all three ethnic groups during the studied period. This is well comparable to other reported studies across the world (Howard, et al., 2001).

In the UK, ethnic disparity in stroke mortality rates has been reported (Saha, et al., 2003). However, highest in-hospital all cause mortality rates in South Asians compared to all other ethnic groups in the current study further demonstrate the vulnerability of this group to the whole spectrum of CVD which had not been reported before (Wolfe, et al., 2006). Long term mortality following a stroke depends on the disease severity as well as other comorbidities which have a direct impact on the recovery. The higher stroke mortality in non Asian populations living in the UK has been mainly attributed to age, hypertension and atrial fibrillation (Rothwell, et al., 2005; Lee, et al., 2007). This has been confirmed in many previous cross-sectional and recent longitudinal studies involving European Caucasian populations (Carter, et al., 2007). The Northern Manhattan Study in the USA also revealed similar risk associations with stroke mortality (Dhamoon, et al., 2007). However, studies from the East Asian countries depict a different scale of risk association. In these studies, the presence of diabetes and hypertension at the time of the event strongly predicted stroke outcomes (Pham, et al., 2007). This clearly suggests a disparity and different distribution of CVD risk factors and their impact on stroke outcomes in the populations in the East when compared to those in the West. However, research into established stroke risk factors has not been thoroughly investigated in the South Asian population. Indeed there is a paucity of longitudinal data demonstrating the impact of these risk factors on South Asian stroke survivors. In addition, data on stroke care provisions in these populations are sparse.

In our five-year mortality analysis, 56% of the South Asian stroke patients were diabetic, and the magnitude of adverse risk that this confers to survival is of particular

concern. Migrant South Asian populations in UK are known to have higher prevalence of diabetes mellitus (McKeigue, et al., 1991; Kain, et al., 2003) which is three- to- nine fold higher than in the Caucasian population (Delu, et al., 2000).

Evidence from migration studies suggests that the change of lifestyle, modifications in dietary habits and improvements in socio-economic status in the migrant South Asian population have resulted in increased development of the macrovascular complications of diabetes (McKeigue, et al., 1991). The reasons for increased long term stroke mortality amongst South Asians with diabetes in the current study are very likely to be linked to poor glycaemic control and lack of modification of life style factors including dietary habits which are proven to be difficult to control in this population (Lanting, et al., 2005). This aspect clearly merits further investigation.

The Whitehall study reported that impaired glucose regulation doubled the risk of death from coronary heart disease among middle aged civil servants (Jarrett, et al., 1986) whereas Chaturvedi reported a similar finding amongst South Asians (Chaturvedi' et al., 1996). In cerebrovascular disease, studies have reported the association between abnormal glycaemic status and adverse stroke outcomes in non-South Asian populations (Ballotta, et al., 2007; Tracey, et al., 1993; Murros, et al., 1992). We are unaware of published long term prognostic data on the impact of diabetes on South Asian stroke patients.

3.4.1: Limitations

There are several limitations to the study analyses, including the hospital-based retrospective registry nature where the results of this study cannot be directly generalisable to all the strokes. The patients who were not admitted to hospital following a stroke but were managed initially in the community may not have been included in the current registry and may have caused a selection bias and under-estimation of the stroke rates. The use of ICD diagnosis codes to select patients with stroke could have resulted in over-estimation of ischaemic stroke rates in this population as a result of classification errors. This was confirmed in the validation analysis where 2.5% of the patients who had a coding for ischaemic cerebral infarction did not have any objective evidence of an ischaemic stroke but had an unspecified stroke. Similarly, exclusion of patients with unspecified strokes may have potentially resulted in under-estimation of the ischaemic stroke rates. However it is very unlikely these errors may have an impact on the comparative analysis of distribution of CVD risk factors amongst studied ethnic groups. Also, the reporting of cardiovascular comorbidities such as peripheral vascular disease and stroke type is limited by the quality of information that is recorded within case notes as a result of recording and reporting errors. Therefore smoking status and alcohol consumption status had not been included in the analysis and may have confounded stroke mortality analysis. Finally, reported prevalence rates in this study are purely based on the hospital stroke admissions, where there could be an under-estimation of the stroke prevalence in the general at-risk population in the community. Indeed patients who had a stroke while receiving in-hospital treatment may have not included in the study and may have influenced the assumptions made on the in-hospital duration of stay.

The reasons for decrease in number of stroke admissions over the studied time period amongst all ethnic groups may not have been due to true reduction of stroke incidence but due to changes in socio-economic and behavioural factors (patients

attending other hospitals for treatment, preferring to obtain community based treatment, migration to other regions) which are in-particular known to exist amongst South Asians. In addition, because this registry was collated retrospectively using an ICD coding for primary diagnosis of ischaemic stroke, we were unable to study South Asian sub groups separately and adjust for other confounding factors.

However it is assumed that all these factors may have equally affected all the concerned ethnic groups and legitimates the comparison of CVD risk factors between the ethnic groups. Moreover, hospital admission rates in this study are also proportional to the prevalence rates reported by Hsu et al (1999) in Leicestershire. Although there has been no change of the ICD 10^{th} revision during the period of our study, threshold levels for diseases such as hypertension have changed. This could result in an over-estimation of the prevalence rates when analysing and interpreting secular trends. Similarly, there could also be an over-diagnosis of strokes on admissions, specifically in older patients, who are admitted with other co-morbidities. Nonetheless, it may not have contributed to the determination of ethnic differences, as one would again assume that changes would apply equally to all cohorts.

The mortality data of the patients who were not admitted to hospital following a stroke but were managed in the community have not been included in the current analysis. This may have caused a selection bias and under estimation of the mortality rates. As a result of the retrospective and coding based nature of this study, we were unable to determine and adjust for other factors including impact of treatment in the Cox regression mortality analysis. Mortality data in the current study refers to all cause mortality, where the underlying specific cause of death was not available for the analysis.

In conclusion, cardiovascular risk profiles among patients admitted with non-haemorrhagic stroke have changed over the last decade. In particular, hyperlipidaemia

has increased, especially among South Asians, where it showed a fivefold increase. The reduced decline in stroke prevalence and 30-day survival of stroke in South Asians in recent years warrants further investigations. Long term stroke mortality in South Asians is associated with presence of diabetes mellitus. This highlights the importance of further prospective & pathophysiological studies to investigate the impact of diabetes on stroke mortality, particularly amongst South Asians.[2,3]

[2]Gunarathne, A., Patel J.V., Hughes, E.A., et al.(2008). Increased five year mortality in the migrant South Asian stroke patients with diabetes mellitus in the United Kingdom: The West Birmingham Stroke Project. **International Journal of Clinical Practice,** 62:197-201

[3]Gunarathne, A., Patel J.V., Hughes, E.A.,(2007).Secular trends in the cardiovascular risk profile and mortality amongst inner city multi-ethnic population in the UK (1997-2005). **Journal of Human Hypertension**,8:2

Chapter 4 : Assessment of arterial stiffness indices in healthy South Asians compared to European Caucasians

Literature review on arterial stiffness and ethnicity (Chapter 1.6.3) highlighted the significant paucity of available data on indices of arterial structure and function amongst healthy South Asians as well South Asians with established cardiovascular or cerebrovascular diseases. The principal objective of this chapter is to firstly determine the structural characteristics of the vessel wall amongst healthy SA compared to that of EC.

4.1 Introduction

South Asians living in the UK have disproportionately higher rates of premature mortality from CVD than the indigenous UK population (Chapter 1). Whilst this excess CVD risk is closely allied with cardiovascular comorbidities that include diabetes mellitus and hypertensive disease (Patel, et al., 2006), the underlying pathophysiology leading to this excess risk remains poorly understood. This increased CVD burden continues to be a source of the disparity in equitable healthcare for this ethnic group. Importantly, there is a markedly earlier progression of disease in South Asians (Gill, et al., 2007), suggesting that the pathogenesis of sub-clinical atherogenesis and premature arteriosclerosis is manifest even in apparently healthy individuals. However, the timely identification of those South Asians at increased risk of CVD is a portentous challenge.

Diabetes and hypertensive disease are both associated with accelerated atherosclerosis and age related arteriosclerosis or ‘vascular ageing’ (Cruickshank, et al., 2002) which are known to cause structural changes in vessel wall characteristics (Laurent, et al., 1994). Importantly, the actions of early initiators of atherosclerosis on the vessel wall are manifested as increased arterial stiffness (Oliver, et al., 2003) which is

accepted as an independent marker of CVD, having prognostic and clinical implications therein (Cruickshank, et al., 2002). In addition, recent evidence supports the utility of arterial stiffness as a surrogate marker of end-organ damage in hypertensive disease (Boutouyrie, 2002).

As previously highlighted, the principal physiological factors that contribute to increased arterial stiffness include age and mean arterial blood pressure (Bramwell, et al., 1922). Mean arterial pressure is defined as the average pressure throughout the cardiac cycle. The mean arterial pressure contributes to vascular changes independent of age and probably has a greater impact on the small-to-medium size muscular arteries, leading to higher peripheral vascular resistance, which can be quantified by analysing pulse wave reflections (Laurent, et al., 2006).

I hypothesised that measures of arterial stiffness would be greater amongst healthy South Asians compared to their European Caucasian counterparts even in the absence of established CVD risk markers. To test this hypothesis, we measured indices of arterial stiffness in apparently healthy South Asians and age- and gender- matched European Caucasians attending a cardiovascular risk assessment clinic over a one year period.

4.2 Methods

4.2.1 Case ascertainment:

Using a stratified sampling approach, hundred volunteers who initially participated in community based cardiovascular risk screening clinics in Sandwell and West Birmingham area (West Midlands, UK) were invited to attend the vascular research clinic (Please see Chapter 2.4.2 for details). Of the initial invited population, 90 South Asians attended the research clinic. Subsequently, using a similar approach age and gender matched 62 European Caucasian healthy volunteers (in keeping with the power

calculations) were recruited for the study. The total cohort (aged 30–75 years) comprised healthy people without any known established CHD risk factors. Participants were excluded if there was any evidence of documented cardiovascular disease or found to be on any regularly prescribed cardiovascular medications. In addition, patients without complete vascular measurements were also excluded from the analysis (8%).

4.2.2 Measurement of arterial stiffness

Arterial stiffness was measured using DVP analysis technique. Please see Chapter 2.5.6 for details of the measurement of arterial stiffness using DVP analysis technique.

4.3 Results

4.3.1 Baseline characteristics:

A total of 90 healthy South Asian volunteers and 62 age and gender matched healthy European Caucasians were recruited for the study (Table 4.1). South Asians and European Caucasians were matched for age, and comparable for body mass index, waist hip ratio, fasting plasma glucose and serum cholesterol. In both groups, the variation of systolic and diastolic blood pressures remained within normal range (<140/90mmHg), although mean arterial pressure levels were significantly higher in the European Caucasians (P =0.048).

4.3.2 Measurements of arterial stiffness:

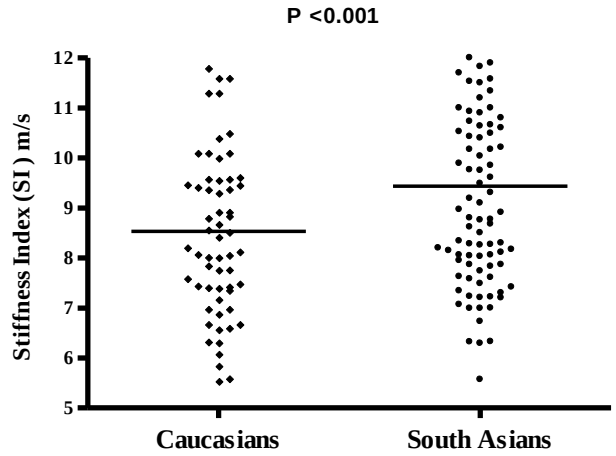
On comparison, South Asians had higher SI compared to European Caucasians (P=0.007) (Figure 4.1). There were no differences in the mean 10 year CHD risk score estimates between South Asians and European Caucasians (South Asians: 6.49(0.76) vs European Caucasians: 7.1(0.7), P=0.54)). SI was strongly associated with the 10 year CHD risk score in both ethnic groups (Pearson correlation (r), South Asians: r=0.65, P<0.001; European Caucasians: r=0.42, P=0.001).

Table 4.1: Baseline characteristics of healthy controls

Risk factor	South Asians (n =90)	Caucasians (n=62)	P Value*
Mean age (years)	45.2(13.4)	46.7(11)	0.13
Males (%)	51.1	50.8	0.26**
Height (m)	164.1(9.9)	170.8(9.3)	<0.001
Body Mass Index (kg/m^2)	25.9(3.2)	27.4(4.5)	0.08
Waist Hip Ratio	0.94(0.08)	0.93(0.08)	0.53
Systolic blood pressure (mmHg)	134.5(12.3)	135.3(15.3)	0.82
Diastolic blood pressure (mmHg)	83.6(9.3)	83.58(10.1)	0.97
Mean arterial pressure (mmHg)	96.9(11.7)	100.8(10.2)	0.048
Serum cholesterol (mmol/l)	4.4(1.4)	4.6(0.72)	0.94
Fasting plasma glucose (mmol/l)	4.9(1.4)	4.5(0.92)	0.43
Stiffness Index (ms^{-1}) (SE)	9.39(0.23)	8.43(0.27)	0.007
Reflective Index	70.9(4.24)	70.6(4.21)	0.61
Current Smokers n (%)	8(8.7)	7(10.4)	0.45
10 year CHD risk score	6.49(0.76)	7.1(0.7)	0.54

Data are mean (SD), n(%).*P values using unpaired t test comparing two groups. ** P values using chi square test Significance level was accepted as p< 0.05 level: CHD: Coronary Heart Disease

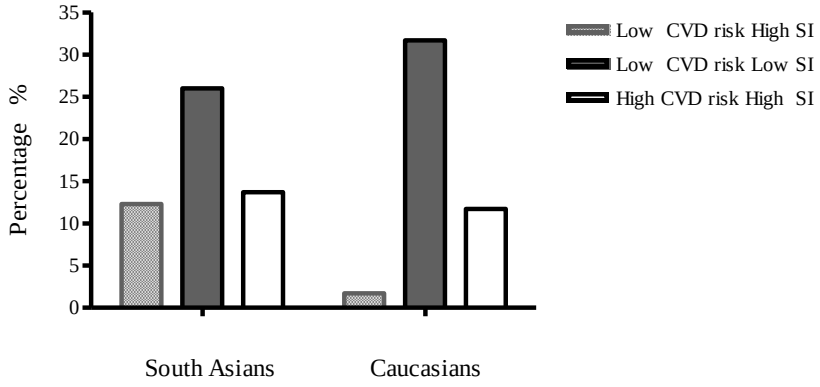
Figure 4.1: Measurements of arterial stiffness (SI) between South Asians and European Caucasians



The ethnic cohorts were combined and stratified into three CHD risk groups: low (<5%), moderate (5-15%) and high risk (>15%).The SI increased in an ordinal fashion from the lowest to the highest risk group (Pseudo R-Square = 0.30; P<0.001). In the

'low CVD risk' category, percentage of the South Asians who had "high SI" was significantly higher than Caucasians (P = 0.04) (Figure 4.1.1)

Figure 4.1.1: Distribution of stiffness index according to CVD risk groups comparing South Asians vs. European Caucasians.

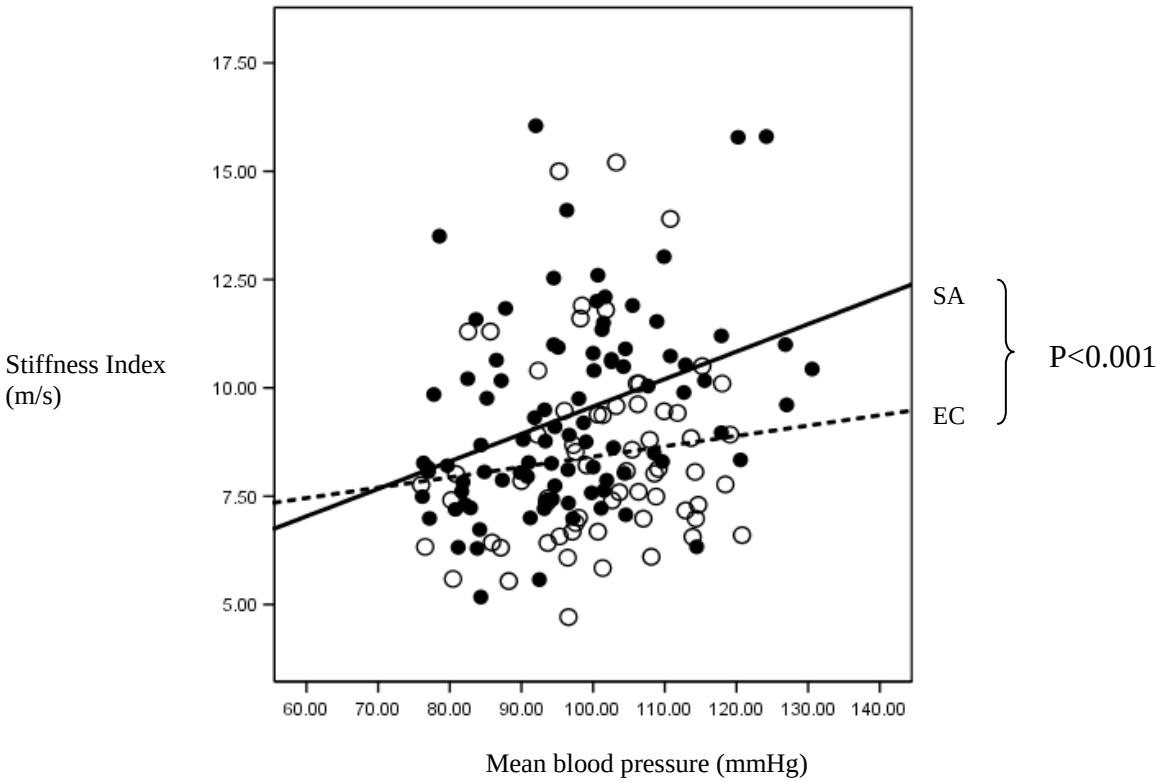


4.4.3 Bivariate and multivariate analysis

In bivariate correlation analysis, age (r=0.43; P<0.001) and mean arterial pressure (r=0.3; P=0.003) were independently related to SI. In sub-group analysis of South Asians, both age and mean arterial pressure levels showed a similar association (Table 4.2). Amongst European Caucasians, there was a significant and positive association between SI and WHR (P = 0.01) and smoking (P= 0.01).

On regression analysis, South Asian ethnicity (Beta (95%CI): 0.31(0.25-0.7); P=0.03)), age (0.07(0.03-0.11); P=0.001) and smoking (1.9(1.68-3.2); P=0.003) were independent predictors of arterial stiffness variation. In South Asians, after adjusting for age and other CVD risk indices, mean arterial pressure independently predicted arterial stiffness (Table 4.2). In a similar analysis of European Caucasians, smoking was an independent predictor of stiffness index.

Figure 4.2: Association between stiffness index and mean arterial pressure



SA = South Asian, EC= European Caucasian; Regression plot co-efficient: SA= Y=3.22+0.06x; EC=Y=6.02+0.02x. P value comparing regression co-efficient (se) of two groups using a formula derived from independent t-test ($t = (b_1 - b_2)/ SE (b_1 - b_2)$.

In linear regression analysis to demonstrate the effect of mean arterial pressure on SI, a significant ethnic difference was found in the slope of the regression (Figure 4.2) for SI vs. mean arterial pressure between South Asians and European Caucasians (P<0.001). These results also demonstrate that for a given mean arterial pressure, South Asians have a higher arterial stiffness even after adjusting for age (Figure 4.2).

Table 4.2: Bivariate analysis (R) and Multivariate analysis (Beta) between stiffness index and CHD risk indices

Risk indices	South Asians				European Caucasians			
	R**	P value*	Beta (95% CI)	P value	R**	P value*	Beta (95% CI)	P value*
Age (years)	0.45	< 0.001	0.11(0.04-0.17)	0.002	0.40	< 0.001	0.05(0.01-0.1)	0. 01
Waist Hip Ratio	0.15	0.15	4.4(1.16-8.8)	0.05	0.32	0.01	3.98(-5.2-8.2)	0.39
Body Mass Index (kg/m^2)	-0.04	0.76	-0.17(-0.3-0.14)	0.38	-0.01	0.89	-0.09(-0.23-0.03)	0.14
Fasting plasma glucose (mmol/l)	0.03	0.71	0.02(-0.67-0.73)	0.93	0.24	0.04	0.27(-0.25-0.8)	0.29
Serum Cholesterol (mmol/l)	0.17	0.11	0.36(.11-0.43)	0.35	0.12	0.51	0.05(0.02-0.22)	0.84
Mean Arterial Pressure (mmHg)	0.34	0.001	0.06(0.01-0.11)	0.01	0.22	0.05	0.01(-0.03-0.06)	0.55
Smoking	0.02	0.78	0.8(-1.9-3.6)	0.52	0.32	0.01	2.11(1.5-3.7)	0.01
Mean heart rate	-0.14	0.23	-0.02(-0.11-0.06)	0.58	-0.15	0.002	-0.04(-0.15-0.07)	0.14

Significance level of the P value was accepted as $p< 0.05$ level

4.4 Discussion

This study is consistent with our hypothesis and previous limited, data (Din, et al., 2006) and demonstrates higher indices of arterial stiffness in an apparently healthy, South Asians compared to the general population. Mean arterial pressure levels in the South Asians were significantly lower compared to the European Caucasians, despite its association with arterial stiffness in the former. Possible explanations for this paradox of higher arterial stiffness despite lower mean arterial pressure levels in South Asians merits careful consideration.

Structural characteristics of both the arterial wall and its function can be altered with exposure to adverse cardiovascular risk indices. In healthy European Caucasians, the extent of the arterial stiffness appears to correlate with conventional CHD risk factor burden (Kalra, et al., 2005). In South Asians, there seems to be a considerable variation in the magnitude of impact of CVD burden on the arterial wall characteristics (Din, et al., 2006). In the current study, South Asians demonstrated to have a greater arterial stiffness for a given blood pressure level after adjusting for other comorbidities including age. It appears that there is an adverse impact of the mean arterial pressure on the vascular system. The reasons for this appear to be multi-factorial, and include genetic susceptibility and the simultaneous presence of other metabolic, inflammatory and oxidative stress related abnormalities (Forouhi, et al., 2001), which are known to have a cumulative impact on the vasculature. Few studies have highlighted the importance of diet and life style changes in South Asians living in Britain compared to the European Caucasian counterparts, which is compounded by transitions in diet and lifestyle on migration to the UK (Patel, et al., 2005). Indeed adverse diet and life style differences observed in migrant South Asians may further augment this unfavourable risk profile (Lean, et al., 2001; Lip, et al., 1996). Such cumulative stressors can reduce

the effectiveness of protective mechanisms thereby allowing pathological processes such as atherosclerosis to become operative, which in turn increases arterial stiffness. Moreover, apparent morphological differences in vessel wall characteristics in South Asians (Lip et al., 1999) may also augment the impact of mean arterial pressure on the vessel wall, resulting in changes in pulse wave form reflections.

The technique and method (i.e. DVP) used to assess arterial distensibility in this study allows examination of the structural integrity of both large and small arteries simultaneously. The observed increase in arterial stiffness in South Asians could, therefore, be attributed to both large and small-to-medium vessel wall abnormalities. Larger proximal artery stiffness is known to cause elevated systolic blood pressure levels (Safar and O'Rourke, 1999) but South Asians are more prone to develop smaller vessel disease (Ajjan, et al., 2007). Hence, normal and comparable systolic blood pressure levels in this South Asian cohort suggest that the observed higher stiffness is more related to smaller and medium vessels abnormalities than to larger vessels.

Despite comparable mean arterial pressure levels (Khattar, et al., 2000), antihypertensive therapy usage (Bhopal, et al., 2002) and changing trends in blood pressure(Lyratzopoulos, et al., 2005) there is still a greater burden of cardiovascular morbidity from hypertension in South Asians in the UK compared to the general population (Khattar, et al., 2000). The pathophysiological basis for this disparity however, has not been explained. Our findings suggest that higher CHD related mortality in South Asians could be related to underlying increased arterial stiffness and associated abnormalities. This highlights the importance of further prospective studies, exploring the possibility of perhaps reducing the thresholds for diagnosis of hypertension in this high risk population as an approach towards prevention of future cardiovascular events and complications of hypertension.

Current guidelines on the cardiovascular risk assessment for people with established cardiovascular disease (CVD) and persons at high risk of developing CVD, do not consider specific risk factor targets for South Asians (JBS2, 2005). A paucity of longitudinal data is likely to hamper an evidence-based consensus approach for ethnically specific CHD management and prevention. As expected, SI is strongly associated with cardiovascular risk scores, in the current study. South Asians had comparable 10 year CHD risk scores to European Caucasians using the ESC risk score model. The data presented highlights a basis for use of arterial stiffness measurements in the early identification of higher risk individuals who are likely to be considered "healthy" and "low risk" in the traditional cardiovascular risk assessment process.

Limitations:

The limitations in the current study include the cross-sectional nature of the design. A large long-term prognostic study would be required to validate the predictive value of arterial stiffness in this ethnic group. In keeping with both the available literature and the taxonomies used in many other studies, we have used the ethnic category 'South Asian'. It is also recognized that classification based purely upon geographical origin masks the considerable heterogeneity in the pathophysiology of the disease amongst South Asians. Furthermore, in the current analysis the clustering impact of other metabolic, inflammatory biomarkers and novel risk indices on the higher arterial stiffness seen in South Asians has not been examined. In addition, the DVP method used to measure arterial stiffness in the current study did not provide information on individual contributions that both large and small arteries make towards wave reflection and overall arterial stiffness. Using a design in which combined methods of measurement (Pulse Wave Velocity and DVP for example) would possibly have provided more comprehensive details thereby giving greater explanatory power to

this study. Finally, the objective of this study was not to measure function of the small vessels by comparing indices of RI pre and post Salbutamol and GTN administration. Therefore, absolute baseline data presented on RI may not necessarily confer any added usefulness.

In conclusion, South Asians were demonstrated to have an increased pulse wave reflection and systemic arterial stiffness compared to European Caucasians, in keeping with our hypothesis. This may be related to an adverse and disproportional impact of the mean arterial pressure on the vascular system. Pathophysiological differences in vessel wall characteristics among South Asians may explain their increased susceptibility to vascular disease and their higher cardiovascular risk.[4]

[4] Gunarathne, A., Patel, J.V., Gammon, B., Hughes, E.A.,(2008). et al.Impact of mean arterial blood pressure on higher arterial stiffness indices in South Asians compared to White Europeans. **Journal of Hypertension**, 26:1420-6.

Chapter 5 : Structural and functional vascular risk indices and related biomarkers amongst South Asian stroke survivors compared to European Caucasian counterparts

5.1 Introduction

Given the higher prevalence of diabetes and hypertension amongst SA compared to EC and impact of diabetes on SA stroke mortality (Chapter 3), the objective of the present chapter is to comprehensively examine the vascular risk indices amongst SA stroke survivors compared to EC, and assess the impact of conventional risk indices on vessel wall characteristics.

Indices of arterial stiffness and endothelial dysfunction are accepted as independent markers of vascular disease, having both prognostic and clinical implications (Perticone, et al., 2001; Laurent, et al., 1994). Established CVD risk factors such as diabetes and hypercholesterolemia are known to alter the underlying vessel wall characteristics (Oliver, et al., 2003) causing impaired endothelial function (Guerci, et al., 2001) and increased arterial stiffness (Cruickshank, et al., 2002), with a consequent increase in greater CVD risk.

The identification of the vessel wall characteristics is important because such characteristics precede changes in haemodynamic parameters (Anderson, et al., 2005) such as mean arterial pressure, which in themselves are strongly linked to adverse cerebrovascular events (Van Bortel, 2002) and provide an insight into the magnitude of the vascular dysfunction (Quyyumi, 1998) even amongst individuals with similar risk factor profiles. Indeed, the extent of the vessel wall damage appears to correlate with

conventional risk factor burden amongst SA (Chapter 3), but only less than a quarter of this excess risk had been explained by the presence of conventional risk factors such as glycaemic status (Chapter 1). A possible explanation may be that clustering effect of inflammatory, oxidative stress and metabolic factors may have an adverse impact on the vessel wall properties directly or synergistically with already established conventional risk factor burdens. Some of the few available studies have previously reported higher prevalence of thrombotic, metabolic and inflammatory (Somani, et al., 2006) markers amongst South Asian stroke survivors; however their impact on the vessel wall characteristics has not been systematically investigated.

SA stroke survivors in the UK have an increased prevalence of diabetes and hypertension (Chapter 3) and it is likely that these established risk indices primarily exert an adverse impact on the endothelium.

Endothelial dysfunction is considered a key stage in the development of atherosclerosis. (Cai, et al., 2000). It is independently associated with cardiovascular events (Al Suwaidi, et al., 2000; Halcox, et al., 2002; Schächinger, et al., 2000; Schächinger,2001) and has been shown to be predictive of the development of future strokes (Holmes, et al., 2003) . People with impaired endothelial function are known to have decreased nitric oxide production (Palmer, et al., 1987). Endothelium-derived nitric oxide is synthesized from the substrate L-arginine via endothelial Nitric Oxide Synthase (eNOS) (Föstermann, et al., 1994; Busse, et al., 1998; Fleming, et al., 2004) and it exerts its cellular mechanisms via cyclic Guanylate Mono Phosphate (cGMP) second messenger interaction (Bauersachs, et al., 1998).

Endothelial cell activation can occur physiologically as a part of host defense mechanisms or pathophysiologically, where homeostasis of the eNOS-NO pathway can

be disturbed by increased expression of chemokines, cytokines and adhesion molecules and reduction in NO bioavailability (Deanfield, et al.,2007). In addition, in the context of cardiovascular risk factors, the cellular environment can switch towards production of reactive oxygen species (ROS) (superoxide or hydrogen peroxide) based on the availability of cofactors such as tetrahydrobiopterin or L-arginine, thereby enhancing oxidative stress (Föstermann, et al., 2006). Asymmetric dimethylarginine (ADMA) which is an endogenously derived competitive antagonist of NO synthase is also increased in these situations (Vallance, et al., 2004). These processes can lead do "eNOS uncoupling" and related endothelial dysfunction (Deanfield, et al.,2007).

NO plays an essential role in maintaining vascular tone, blood pressure (Ohashi, et al., 1998) and lower levels are thought to be involved in atherogenesis (Wolf, et al., 1997). Given that the majority of endothelium-derived NO production depends on eNOS concentration, reduced availability of eNOS also exerts a similar impact on the vascular homeostatic mechanisms (Feng, et al., 2002). The dysfunctional eNOS-NO pathway is considered to be an important early indicator of cardiovascular risk amongst higher risk populations (Hare, 2004). Patients with impaired endothelial function are known to have increased risk of cardiovascular disease (Rossi, et al., 2003) including strokes (Corrado, et al., 2008) and reduce NO bioavailability and eNOS expression has been well demonstrated with many other stroke populations (Cheng, et al., 2008;Kim, et al., 2007). However there are limited data examining the molecular interactions of endothelially-derived nitric oxide production amongst the South Asian stroke population and their influence on vessel wall characteristics.

The **first** objective of the present study was to test the hypothesis that indices of arterial stiffness and endothelial dysfunction are higher in SA stroke survivors compared

to European Caucasians(EC), and that conventional risk indices may able to explain these vessel wall abnormalities.

With the aim of investigating the adverse impact of eNOS-NO axis determinants on underlying vessel wall characteristics amongst South Asian stroke survivors, **secondly** I hypothesised that the endothelium derived NO, eNOS and cGMP levels are significantly lower amongst South Asian stroke survivors compared to their European Caucasian counterparts and that the reduced availability of nitric oxide is associated with greater arterial stiffness and impaired endothelial function.

To explore the adverse clustering effect of inflammatory, oxidative stress and metabolic factors on the vessel wall properties, **thirdly** I hypothesised that the levels of markers of inflammation (Interleukin (IL6), High sensitivity C-reactive protein (hsCRP), Tumour necrosis factor (TNF alpha)), oxidative stress (oxidised Low Density Lipo-protein (oxLDL), Haem-Oxygenase(HO), endothelial activation and thrombosis (von Willebrand Factor (vWF)) and metabolic status (Apo lipo protein (Apo A), Apo lipo-protein B(Apo B)) are different amongst South Asian stroke survivors and aberrant levels are associated with increased arterial stiffness and impaired endothelial function.

5.2 Methods

5.2.1 Case ascertainment

South Asian stroke patients with a diagnosis of ischaemic stroke, who were initially admitted to Sandwell and West Birmingham NHS Trust (Sandwell and City Hospitals) between 2005 and 2007, were invited to participate in the study after three months of the initial presentation (Please see Chapter 2.4.2.2 for details). SA were defined by self-reported ethnicity as being Indian, Pakistani, Sri Lankan, Nepalese, and Bangladeshi, and whose grandparents (at least three generations) also originated from the

Indian subcontinent (Chapter 1.2). Of the responders, the first consecutive 100 patients who attended the initial screening visit were prospectively recruited for the vascular sub study. Based on the power calculations (for vascular measurements) at least 58 people were required in each group. Therefore using the same stroke unit database , age, gender and post code (same area) matched Caucasian stoke patients who were admitted during the same period were invited and of the responders, the first attended 60 patients were recruited for this vascular sub study. After excluding the patients who had died or who developed a second stroke with in the first three months period, 28% of the South Asian and 6% of the Caucasian stoke patients who originally invited and agreed to participate in the study did not attend the subsequent screening visit. Four patients who didn't have complete vascular measurements were excluded from the final analysis.

5.2.2 Measurement of Arterial Stiffness & Endothelial function:

Arterial stiffness & endothelial function was measured using the Digital Volume Pulse analysis (DVP) technique. Please see section 2.5.6 for details.

5.2.3 Quantitative determination of biochemical parameters

Blood samples were collected after three months from the acute event and were taken following an overnight fast, on the same day of the vascular measurements. Blood samples were prepared at the Laboratory for Clinical Biochemistry Research, Sandwell and West Birmingham NHS Trust. Biomarkers (IL6, TNF alpha, oxLDL, HO, E-Selectin, vWF, NO, eNOS, cGMP) were measured by 2-site enzyme linked immunosorbent assay, with commercially available antibodies from R&D systems (UK) and Mercodia (USA). HsCRP, Apo AI, Apo B, Serum Cholesterol and fasting glucose levels were measured using an auto analyser (Cobas Integra 400, Roche, Switzerland). Please see section 2.5.8 for details.

5.3 Results

5.3.1 Baseline characteristics:

Of the cohort of 289 individuals, SA stroke survivors (70.4 % male; mean age 62.6 SD (13) years) were initially compared to age and gender matched 60 EC counterparts (Table: 5.1, 5.2). The majority of the SA stroke survivors were of Indian origin (67.4%). Both ethnic groups were comparable for cardiovascular risk profile, except for the higher prevalence of diabetes mellitus in SA (47.4% vs. 25.1%; P=0.007) and atrial fibrillation in EC (13.3% vs. 3.1%; P=0.04). Among SA there was a lower prevalence of alcohol and tobacco consumption (P<0.01) compared to EC. The majority were on anti-diabetic (SA: 48.4% vs. EC: 26.8%, P=0.001) lipid-lowering (SA: 88.7% vs. EC 94.6%) and anti-hypertensive (SA: 86% vs. EC: 86%) medications. The use of warfarin (P=0.04) and dipyridamole (P=0.03) was higher amongst EC stroke patients, whilst there was a greater use of sulfonylureas (P<0.001) amongst SA stroke survivors.

5.3.2 Radiological imaging:

Of the total cohort, 9.5% of the stroke patients with clinical symptoms of stroke had normal CT scans, without any evidence of ethnic variation. Majority of SA as well as EC had radiological evidence of fronto-parietal infarctions and did not demonstrate any ethnic differences (P=0.69). The majority of the SA had normal duplex scan results (76.3% vs. 36.7%; P<0.001). Of the patients with abnormal scan findings, 20.1% EC demonstrated evidence of significant carotid artery stenosis (>50%) (Table 5.2). According to the Bamford and TOAST criteria, SA had higher small vessel (29.6 Vs 15.5; P=0.04) and lacunar infarctions (32.6 vs. 13.3; P=0.01) compared to EC.

Table 5.1: South Asians vs. European Caucasians with established cardiovascular risk indices

	SA Stroke (n=97)	**EC Stroke** (n=60)	**SA Risk** (n= 59)	**SA Healthy** (n=73)	**P value***	**P value****
Age	62.6(13)	64(8)	65.2(9)	50(15)	0.91	<0.001
Fasting plasma glucose (mmol/l)	6.2(1.3)	4.1(0.9)	3.1(1.7)	2.6(1.4)	0.57	<0.001
Fasting plasma glucose (mmol/l) Diabetics	7.2(0.9)	5.6(1.2)	3.8(1)		<0.001	<0.001
Fasting plasma glucose (mmol/l) Non Diabetics	5.3(1)	3.6(0.6)	3.4(0.8)	3.3(0.7)	<0.001	<0.001
Body mass index (Kg/m^2)	27.7(4.6)	28.8(5.8)	27.9(3.8)	26.2(3.2)	0.02	0.03
Waist hip ratio	0.95(0.1)	0.94(0.1)	0.93(0.1)	0.91(0.1)	0.23	0.9
Mean Blood pressure (mmHg)	100.7(12)	110.5(13)	107(11)	100.5(13)	0.9	0.21
Total Cholesterol (mmol/l)	3.8(0.5)	3.7(0.7)	4.4(0.7)	4.2(0.8)	0.001	<0.001
LDL (mmol/l)	1.7(0.7)	1.8(0.7)	2.7(0.9)	2.5(0.5)	0.75	<0.001
HDL (mmol/l)	0.92(0.3)	0.98(0.2)	1.0(0.3)	0.99(0.3)	0.74	0.02
Mean Systolic pressure (mmHg)	142.3(21)	135.79(20)	141.2(15)	129.4(16)	0.9	0.02
Mean Diastolic pressure (mmHg)	79.7(11)	79(12)	84.4(11)	82(12)	0.8	0.05
Heart rate (per min)	69.2(15)	68.4(11)	70.4(10)	69.5(9)	0.83	1
Arterial Stiffness (SI)m/s	11.2(2)	9.7(2)	10.1(2)	9.3(2)	<0.001	<0.001
Endothelial function (Δ RI) %	4.3(0.3)	7.9(0.6)	7.2(0.3)	10.2(0.5)	0.001	<0.001

P* comparing SA stroke survivors vs. EC stroke survivors, significance at P<0.05 levels using unpaired t-test, P** comparing across all groups, significance at P<0.05 levels using one way ANOVA

Table 5.2: Baseline characteristics comparing South Asians vs. Europeans

	South Asian Stroke	European Caucasian Stroke	P Value
Male	70.1	65.1	0.49
Ethnicity			
Indian	67.4		
Pakistani	22.1		
Bangladeshi	10.5		
CVD risk profiles			
Myocardial infarction	15.5	15	0.51
Ischaemic heart disease	28.6	11.7	0.08
Hypertension	68	85	0.15
Diabetes	47.4	25.1	0.007
Atrial fibrillation	3.1	13.3	0.04
Heart failure	2.1	5	0.45
Hyperlipidaemia	86.6	93.3	0.07
Endarterectomy	3.1	11.7	0.07
Peripheral vascular disease	2.1	3.3	0.78
Coronary artery bypass graft	7.2	5	0.58
Family history of MI	16.5	28.3	0.39
Family history of Stroke	18.6	21.4	0.6
Smoking	13.4	41.7	0.01
Alcohol	16.5	57.9	0.001
CVD treatment			
Hypertension treatment	85.9	86	0.49
Diabetes treatment	48.4	26.8	0.001
Cholesterol treatment	88.7	94.6	0.79
ACEI	50.5	50	0.175
Beta Blocker	18.6	23.3	0.97
Calcium Channel Blocker	21.6	20	0.34
Loop Diuretics	8.2	13.3	0.57
Thiazide Diuretics	11.3	15.6	0.51
Angiotensin receptor blocker	9.3	5	0.17
Alpha Blocker	3.1	11.7	0.07
Aspirin	63.9	80	0.94
Ticlodipine	1.3	1	0.37
Dipyridamole	4.1	36.7	0.03
Clopidogral	13.4	6.7	0.07
Warfarin	4.1	15	0.04
Fibrates	1	6.7	0.06
Insulin	12.4	13.3	0.73
Sulphonyl urea	21.6	3.3	<0.001
PPRA γ	5.2	3.3	0.41
Biguanides	25.8	20.8	0.11

P value comparing SA vs. EC significance at P<0.05, ACEI: Angiotensin Converting Enzyme Inhibitor; CVD:Cardiovascular Disease

Table 5.3: Stroke characteristics comparing South Asians vs. European Caucasians

	South Asian Stroke	European Caucasian Stroke	P Value
Bamford classification			
TACI	10.1	15	0.34
PACI	27	36.7	0.08
LACI	32.6	13.3	0.01
POCI	6.7	11.7	0.17
Unspecified	23.6	23.3	0.29
TOAST classification			
Large vessel disease	16.7	27.6	0.04
Small vessel	29.6	15.5	0.03
Cardio embolic	1.9	8.6	0.18
Un specified	51.8	48.3	0.68
Clinical Motor involvement			
Hemi paresis	69.1	68.3	0.89
Para paresis	1.8	1	0.78
Mono paresis	9.1	10	0.92
Clinical Speech involvement			
Aphasic	19.6	13.3	0.26
Nominal	1.8	1	0.89
Expressive	16.1	36.7	0.04
Cranial Nerve involvement	52.7	62.7	0.18
Incontinence	20.4	13.3	0.31
CT diagnosis			
Normal	12.3	6.7	0.35
Infarction	71.4	81.7	0.54
Age specific changes	0	1.7	0.67
Not specified	16.3	9.9	0.23
CT area			
Fronto parietal	42.9	43.3	0.67
Temporal	4.3	1.7	0.56
Internal capsule	8.7	6.9	0.78
Cerebellar	6.5	6.9	0.88
Basal ganglion	30.4	19.0	0.08
Un specified	13	3.4	0.15
Duplex Scan			
Normal	76.3	36.7	<0.001
>50% stenosis*	2	20.1	<0.001
<50% stenosis	11.8	26.7	0.003
unspecified	9.9	16.5	0.01

TACI: Total Anterior Circulation Infarct, PACI: Partial Anterior Circulation Infarct, POCI: POsterior Circulation Infarct, LACI:LACunar Infarct.*P value comparing SA vs. EC significance at P<0.05, * Classified according to Doppler velocities and NASCET criteria (Barnett, et al., 1991)

In EC, 27.6% of the strokes were due to cerebral large vessel disease compared with 16.7% amongst SA (P=0.04) (Table 5.2).

5.3.3 Stroke subjects compared to risk factor and healthy controls

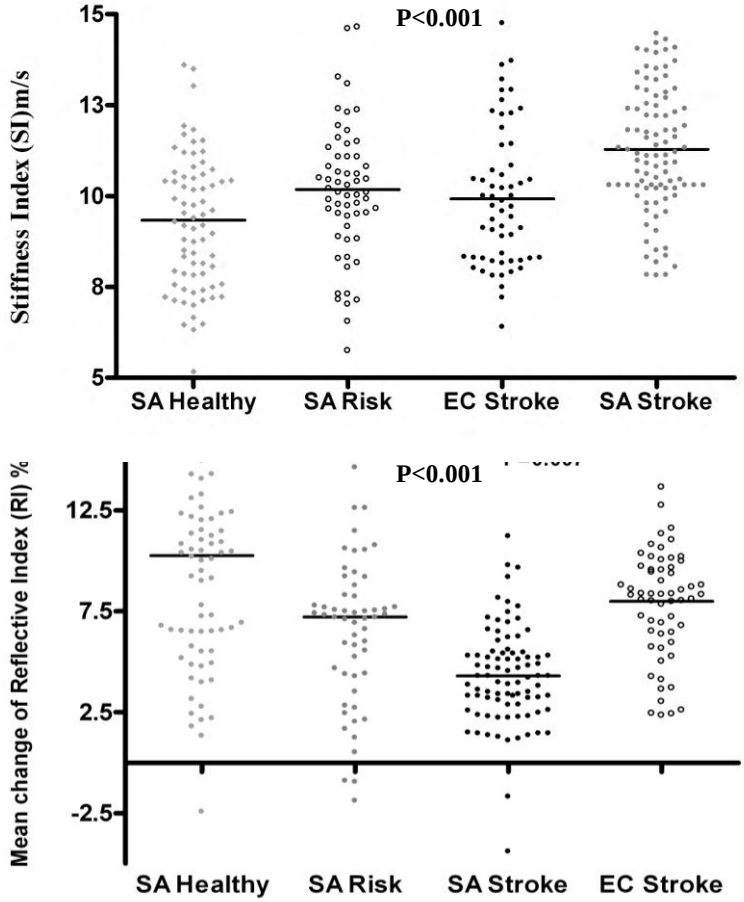
SA stroke survivors had significantly elevated serum cholesterol and plasma glucose levels as well as body mass index (BMI) compared to EC (P<0.05). Blood pressure parameters were comparable. As expected, compared to risk and healthy SA controls, stroke survivors had significantly higher risk indices (P<0.05) but similar waist-to-hip ratio measurements (Table 5.1)

5.3.4 Measurements of arterial stiffness and endothelial function

Indices of arterial structure and function were available for 277 (South Asian, 217; European Caucasian, 60) individuals. Of the total cohort, arterial stiffness measurements (SI) were significantly higher in SA stroke patients compared to EC (P<0.001) (Figure 5.1). Compared to healthy controls, all other groups had significantly higher SI (P<0.001; Table 5.1). In post hoc analysis, comparing SA stroke patients vs. SA risk factor controls, SI remained significantly higher amongst SA stroke patients (P=0.002).

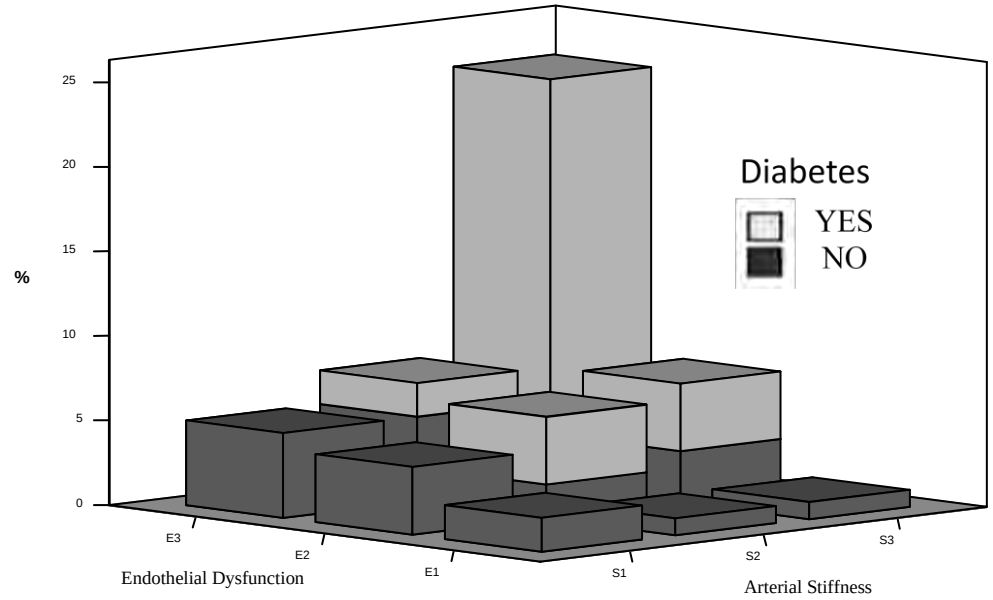
The endothelium-dependent vascular function of each individual was calculated by determining the relative change in RI (Δ RI %) post-salbutamol inhalation. SA stroke patients had significantly poorer endothelial function when compared to EC (P<0.001) (Figure 5.1). As expected, SA healthy controls had significantly better endothelial function compared to groups with risk factors (P=0.001). Analysis of the SA stroke patients according to SI and Δ RI % tertiles, revealed that the majority of diabetic SA stroke survivors had the highest arterial stiffness and poorest endothelial function (Figure 5.2)

Figure 5.1: Measurements of arterial stiffness and endothelial function comparing South Asians vs. European Caucasians



A, Measurements of arterial stiffness (SI) among SA vs EC stroke patients.. **B**, Measurements of endothelial function (RI) among South Asian (SA) vs. European Caucasian (EC) stroke patients *P* value using 1-way ANOVA comparing vascular risk indices SA vs. EC stroke patients.

Figure 5.2: Distribution of endothelial function and arterial stiffness according to tertiles



Endothelial function tertiles (Δ RI) %: E1: > 8.8, E2:5.3-8.8, E3: <5.2, Arterial Stiffness tertiles (ms^{-1}): S1 :< 9.1, S2:9.2-11.1, S3 :> 11.2

5.3.5 Correlations and multivariate regression

On univariate analysis, there was a significant negative association (r) between SI and Δ RI (r= -0.28; P<0.001) and SI and age (r=0.27, P<0.001) for the total population. SI was significantly associated with fasting plasma glucose (r=0.45, P<0.001), total cholesterol (r=0.24, P<0.001), WHR (r=0.3, P<0.001) and mean arterial pressure (r=0.2, P=0.002) amongst SA and with a total cholesterol level (r=0.3, P=0.01) amongst EC. There was a negative association between endothelial function and fasting glucose (r=-0.4, P<0.001), total cholesterol (r=-0.18, P=0.03), BMI (r=-0.2, P=0.007) and WHR (r=-0.17, P=0.02) amongst SA but no significant association was apparent amongst EC stroke survivors.

To determine the factors influencing arterial stiffness and endothelial function, stepwise multiple linear regression analyses were performed. Conventional cardiovascular risk factors: diabetes status, hypertension, hypercholesterolemia, atrial

fibrillation, smoking, higher waist hip ratio, higher age (0=No, 1=Yes), gender (male: 1, female: 2) and ethnicity (SA: 1, EC: 2) were included in the models as independent variables. Hence, SA ethnicity was introduced to the model as a negative variable compared to EC (SA=1 vs. EC=2). In keeping with the original hypothesis, it is expected therefore to obtain a "positive" Beta value for endothelial function and a "negative" value for higher arterial stiffness for SA ethnicity.

The results of the regression analysis are demonstrated in table 5.4. The Beta value for SA ethnicity was "positive" and statistically significant for endothelial function, indicating an independent association between impaired endothelial function and SA ethnicity whereas the Beta value for SA ethnicity was "negative" and significant for arterial stiffness demonstrating an association between higher arterial stiffness and SA ethnicity. Amongst SA, diabetic status independently associated with both arterial stiffness and endothelial function. In interaction analysis, SA ethnicity and diabetic status together had a significant association with arterial stiffness (F=6.4, P=0.02) but not with endothelial function (F=1.5, P=0.22) (Figure 5.3). The strength of the association of these variables together did not demonstrate any improvement when statistically compared to their individual associations (Table 5.5). In these models, only 25% of the endothelial function abnormalities and 31% of the arterial stiffness aberrances were explained by the presence of traditional risk factors amongst SA.

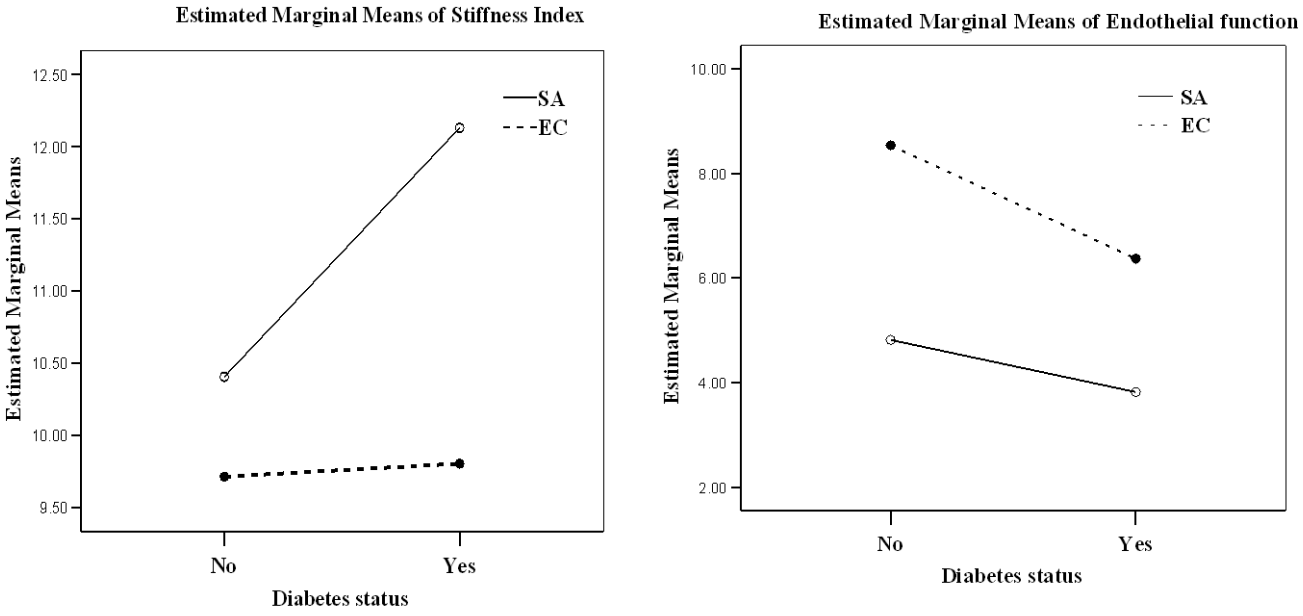
Table 5.4: Multivariate analysis demonstrating independent predictors of arterial stiffness and endothelial function

Group	Predictors	Beta	SE	P value
Endothelial function(Δ RI) %				
Stroke Cohort	South Asian ethnicity	3.2(0.8-4)	0.5	0.001
(R^2 = 0.36)	Diabetic status	-1.4(-3.1- -0.9)	0.4	0.004
Total South Asians	Diabetic status	-2.9(-3.5 - -0.5)	0.7	0.001
(R^2 = 0.25)	Hypercholesterolemia	-3.2(-4 - -0.9)	0.8	0.001
European Caucasians	Diabetic status	-2.2(-3.8 - 0.06)	0.9	0.02
(R^2 = 0.15)				
Arterial stiffness(SI)				
Stroke Cohort	South Asian ethnicity	-1(-1.7- -0.3)	0.3	0.002
(R^2 = 0.24)	Diabetic status	1.1(0.5-1.7)	0.3	0.001
Total South Asians	Age	0.02(0.006-0.04)	0.009	0.01
(R^2 = 0.31)	Waist Hip Ratio	4.5(1.9-7)	1.3	0.001
	Diabetic status	1.3(0.8-1.9)	0.2	0.001
	Hypercholesterolemia	0.6(0.1-1.1)	0.2	0.02
European Caucasians	Male gender	-2(-3.6- -0.7)	0.6	0.004
(R^2 = 0.25)				

Table 5.5: Interaction analysis of diabetes status and ethnicity demonstrating strength of association between arterial stiffness and endothelial function

Variables	F value	P Value
Arterial Stiffness (SI)		
SA Ethnicity	22.003	0.000
Diabetes	7.9	0.005
SA Ethnicity* Diabetes	6.458	0.012
Endothelial Function (RI)		
SA Ethnicity	43.54	0.000
Diabetes	11.07	0.001
SA Ethnicity* Diabetes	1.51	0.22

Figure 5.3: Interaction analysis of diabetes status and ethnicity demonstrating strength of association between arterial stiffness and endothelial function



On an exploratory analysis according to previously reported cut-off points by our group (Chapter 2) and others (Rambaran, et al., 2008), the total group was classified as having poor, average and good endothelial function and arterial stiffness (Table 5.6). Of the stroke population 61.3% of the SA patients demonstrated to have higher arterial stiffness (SI :> 11 ms^{-1}) compared to 23.3% of the EC (P<0.001). Similarly 57.9% SA stroke survivors had poor endothelial function (Δ RI: < 5%) compared to 15% of the EC.

Table 5.6: Arterial stiffness and endothelial function tertiles comparing South Asians vs. European Caucasians

Vessel wall characteristics		**Total** 289 (%)	**SA** 229 (%)	**EC** 60 (%)	**P value***
Endothelial function	Poor(<5)	31.1	38.7	15	<0.001
Δ RI(%)	Average (5-10)	43.8	38.2	63.3	
	Good (>10)	25.1	23.1	21.7	
Arterial stiffness	High (>11)	34.6	35.4	23.3	0.05
SI(ms^{-1})	Medium (9-11)	32.7	39.3	36.7	
	Normal (< 9)	32.7	25.3	40	

5.3.6 Analysis of indices of eNOS-NO axis

Levels of eNOS, NO and cGMP were available for 289 participants and were non-parametrically distributed, with median values (inter-quartile range) of 1.5(0.7-3.5), 37.9(18-74) and 111(76-149) respectively.

5.3.6.1 Relationship to clinical demography and associated comorbidities

eNOS levels were significantly lower among SA (P=0.001), stroke patients (P=0.04) and hypertensive persons (P=0.04). Other differences in eNOS levels according to clinical features (age, smoking status, wait hip ratio, BMI, diabetes status) did not reach significance. Levels of NO were significantly lower in SA (P=0.001), diabetics (P=0.001), hypercholesterolemics (P=0.02) and patients with strokes (P=0.003). cGMP levels were lower amongst SA (P=0.001). SA stroke patients had significantly lower levels of eNOS, NO and cGMP levels compared to EC stroke patients (P=0.001) and SA controls (P=0.001) (See Table 5.7 and Figures 5.4 and 5.5).

Table 5.7: Indices of eNOS-NO axis comparing South Asian vs. European Caucasians

	SA Stroke (n=97)	**EC Stroke (n=60)**	**SA Risk (n=59)**	**SA Healthy (n=73)**	**P value***	**P value****
e-NOS (ng/ml)	0.8 (0.4-1.3)	4 (2-9)	1.3 (0.7-2.2)	2.5 (1.1-5.3)	<0.001	<0.001
cGMP (pmol/ml)	84.2 (62-130)	137 (107-189)	118 (93-148)	107 (71-139)	<0.001	<0.001
Nitrate (umol/l)	20.7 (10-42)	50 (28-99)	40.6 (21-76)	59.3 (21-102)	<0.001	<0.001

**P value using Kruskal Wallis test comparing differences across all four groups, * P value using Mann Whitney U test*, comparing SA stroke vs. EC stroke.

Figure 5.4: eNOS and Nitrate concentrations comparing South Asians vs. European Caucasians

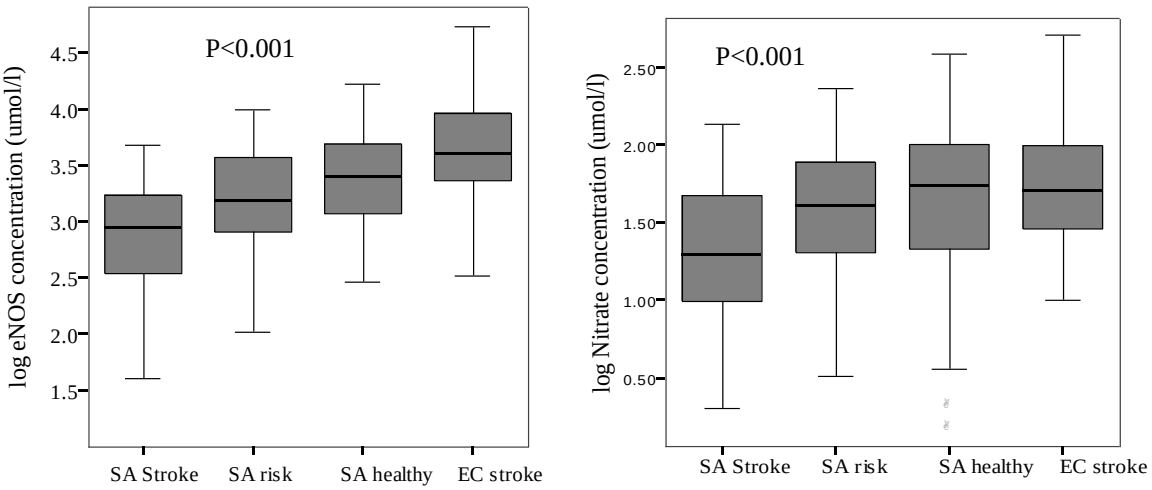
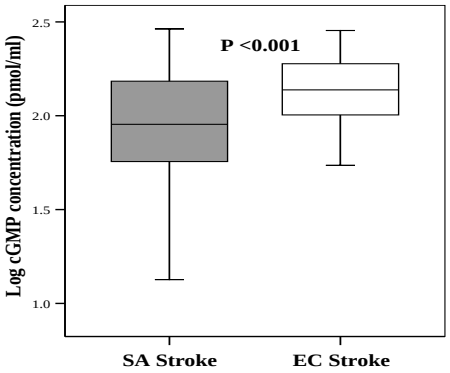


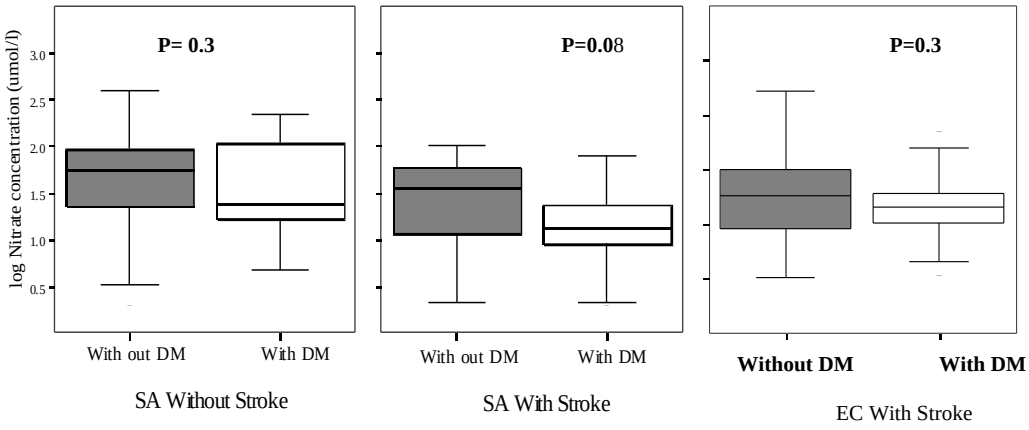
Figure 5.5: cGMP concentrations comparing South Asians vs. European Caucasians stroke survivors.



P value using one way ANOVA comparing differences across all four groups and using independent t-test comparing SA stroke vs. EC stroke.

In a separate analysis, SA with diabetes (total) were demonstrated to have significantly lower NO levels compared SA without diabetes (P=0.001) and EC stroke patients with diabetes (P=0.002). This difference was also apparent but non- significant amongst SA with stroke (Figure 5.6). Similarly, eNOS levels were significantly lower (P<0.001) amongst SA with hypertension compared to SA healthy controls.

Figure 5.6: Nitrate concentration in both ethnic groups comparing patients with and without diabetes

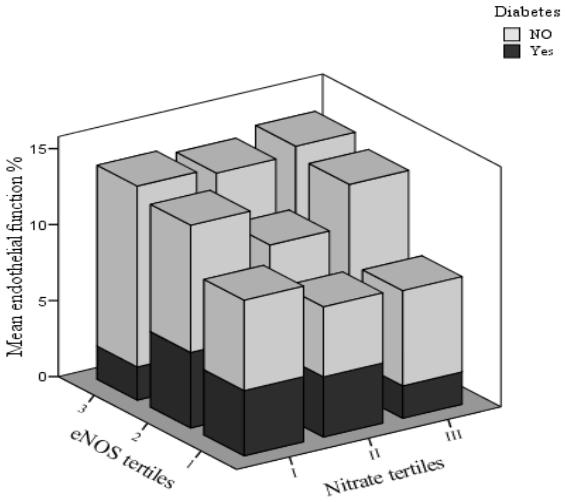


P values using independent t-test comparing patient with and without diabetes. Significance at P<0.05 level.

5.3.6.2 Relationship to arterial structure and function

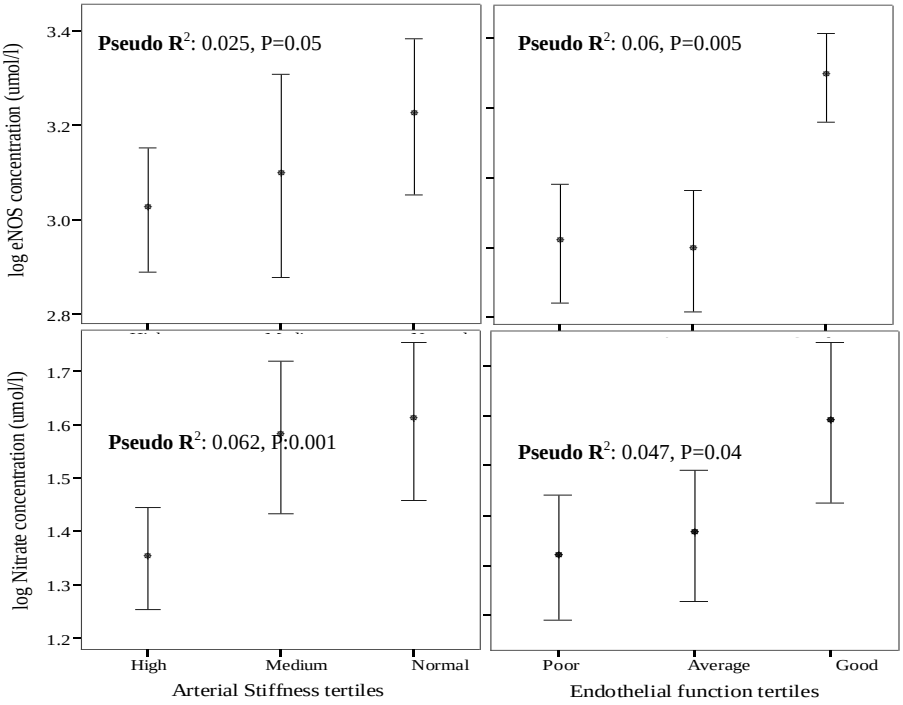
With respect to the arterial stiffness and endothelial function tertiles, SA with higher arterial stiffness and relatively poor endothelial function had significantly lower eNOS and NO levels (P<0.05). This significance was not apparent in the cGMP measurements. In sub-group analysis, SA with higher NO and eNOS levels (tertile: 3) were demonstrated to have better endothelial function compared to lower NO and eNOS concentrations. This difference was more significant amongst people without diabetes (Figure 5.7). In ordinal regression analysis NO and eNOS increased in an ordinal fashion with arterial stiffness (Pseudo R^2: NO:0.04, P:<0.001, eNOS:0.06, P=0.01), and endothelial function (Pseudo R^2: NO:0.06, P:<0.001, eNOS:0.11, P<0.001) tertiles respectively (Figure 5.8).

Figure 5.7: Mean endothelial function amongst South Asians according to eNOS and Nitrate tertiles and diabetic status



eNOS tertiles: 1: (<2.9), 2(3-3.4), 3: (>3.4), NO tertiles: I:(<1.2), II:(1.3-1.7), III:(>1.8)

Figure 5.8: Distribution of NO and eNOS concentrations (95% CI) amongst South Asians according to tertiles of arterial stiffness and endothelial function



P values using ordinal regression across tertiles of arterial stiffness and endothelial function. Significance at P<0.05 level. Error bars represent 95% CI.

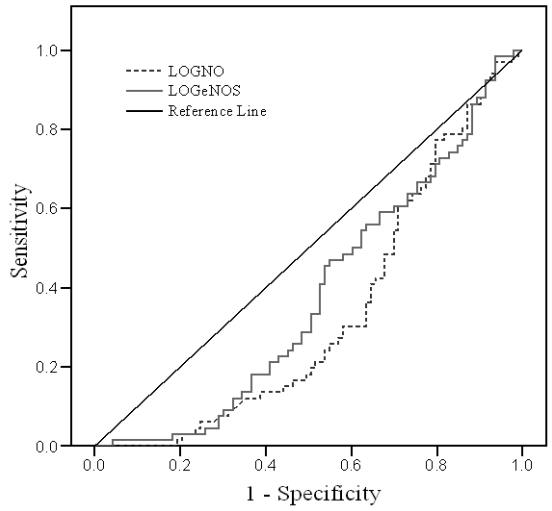
5.3.6.3 Correlations and Multivariable Regression

On univariate analysis there was a significant association between NO and arterial stiffness (r:-0.21, P=0.001). NO level was also associated positively with endothelial function (r=0.2, P=0.001). There was a positive association between eNOS level and endothelial function (r=0.3, P=0.001). In a separate analysis eNOS levels significantly positively associated with NO (r=0.2, P=0.002). In multivariate regression analysis, after adjusting for age and other cardiovascular co-morbidities, lower NO and eNOS levels were negatively associated with increased arterial stiffness [Beta(95%CI):-NO: -0.46(-0.0.9- -0.006), P=0.04; eNOS:-0.5(-0.1- -0.01), P=0.009]. Independent associates for lower NO levels amongst SA were diabetes [Beta(95%CI):-.2.6(-0.3- -0.05), P=0.009] and hypercholesterolemia [Beta(95%CI):-2.7(-0.3—0.06, P=0.006). Independent associates for lower eNOS levels were hypertension [Beta(95%CI):-.2.2(-0.3- -0.02), P=0.02] and hypercholesterolemia [Beta(95%CI):-3.1(-0.4- -0.09, P=0.002) respectively.

5.3.6.4 Receiver Operator Characteristic (ROC) curve analysis

ROC analysis was performed to asses the discriminatory utility of eNOS, NO and cGMP levels in identifying subjects with higher arterial stiffness and poor endothelial function. ENOS and NO levels were found to be the most useful variables in the SA population. NO level had a better discriminatory utility to identify patients with higher arterial stiffness [Area Under Curve (AUC) :0.67(SE 0.04), P=0.001] (Figure 5.9) whereas eNOS was appeared to have the greater utility as a marker in discriminating people with poor endothelial function [AUC:0.63(0.04),P=0.001] compared to NO[AUC :0.62(0.05),P=0.003], and cGMP[AUC:0.51(0.06),P=0.11] levels.

Figure 5.9: **Receiver Operating Curve analysis of eNOS-NO axis markers discriminating higher arterial stiffness amongst SA.**



NO: (AUC:0.67(0.03),P=0.001)

eNOS: (AUC :0.60(0.03), P=0.01)

*P value using chi square comparing SA vs. EC, significance at <0.05 level. SA: South Asian, EC: European Caucasian

5.3.7 Analysis of Inflammatory indices

Serum hsCRP, TNF and IL6 levels were available for 289 individuals (Table 5.8). HsCRP levels were significantly higher amongst females (P=0.05), subjects with higher BMI (P<0.001) and strokes (P=0.001) but without any ethnic variation. SA stroke patients had significantly higher hsCRP levels compared SA controls (P=0.01) (Table 5.8). TNF level was significantly higher amongst SA stroke patients compared to EC (P<0.001) and controls (P<0.001). Both hsCRP levels and TNF levels were significantly higher in South Asians with impaired endothelial function and increased arterial stiffness (<0.05), (Tables 5.9 and 5.10). In bivariate analysis TNF alpha levels demonstrated a weak association with SI (r=0.16, P=0.01) and Δ RI (r= -0.16, P=0.02).

IL6 level was significantly higher amongst SA compared to EC (P=0.001). SA stroke patients had significantly higher IL6 level compared to EC stroke patients (P=0.001) and SA controls (P=0.001), (Table 5.8). However IL6 level was comparable across endothelial and arterial stiffness tertiles in both ethnic groups.

Table 5.8: Indices of inflammation, oxidative stress, thrombosis and dyslipidaemia comparing South Asian vs. European Caucasians

	SA Stroke (N=97)	EC Stroke (n=60)	SA Risk (n=59)	SA Healthy (n=73)	P value*	P value**
Markers of inflammation						
hsCRP(mmol/l)	2.4(1-6)	1.9(1-7)	1.6(1-3)	1.3(1-3)	0.3	0.01
TNF α (pg/ml)	162.6(104-215)	67.6(33-126)	108.5(72-153)	95.5(69-156)	<0.001	<0.001
IL6 (pg/ml)	44.6(23-61)	25.1(14-32)	34.6(16-45)	28.1(17-40)	<0.001	<0.001
Markers of endothelial activation & thrombosis						
E-Selectin (ng/ml)	37.5(21-56)	3.3(1-12)	17.7(13-24)	17.6(13-26)	<0.001	<0.001
vWF(u/dl)	109.4(98-120)	106.9(88-129)	130(117-140)	102(83-130)	0.65	<0.001
Markers of oxidative stress						
Oxidised LDL(u/l)	60.2(36)	41.4(21)	44.5(24)	56.1(22)	0.01	<0.001
HO(ng/ml)	9.7(8-11)	9.2(6-10)	6.9(6-9)	6.7(5-8)	0.02	<0.001
Markers of dyslipidaemia						
Apo A (mmol/l)	1.2(0.2)	1.4(0.2)	1.9(0.1)	2.0(0.2)	<0.001	0.03
Apo B (mmol/l)	0.44(0.1)	0.59(0.1)	0.64(0.1)	0.68(0.2)	<0.001	<0.001
Apo B/A	0.3(0.1)	0.4(0.1)	0.3(0.2)	0.3(0.1)	0.2	0.08

**P value using Kruskal Wallis test comparing differences across all four groups, * P value using Mann Whitney U test*, comparing SA stroke vs. EC stroke, Significance at <0.05 level, FPG: Fasting Plasma Glucose

Table 5.9: Indices of inflammation, oxidative stress, and thrombosis, according tertiles of arterial stiffness amongst South Asians

	South Asians (n=229)		
	High	**Normal**	**P value**
hsCRP (mmol/l)	2 (0.9-4.9)	1.3(0.7-3.4)	0.05
IL6 (pg/ml)	37.1(21-60)	31.7(19-43)	0.2
TNF (pg/ml)	147(95-229)	109(70-180)	0.02
VWF (u/dl)	112(100-134)	103(81-130)	0.03
E-Selectin (ng/ml)	21.1(14-48)	18.5(13-27)	0.08
HO (ng/ml)	8.6(6.3-11)	7.2(5.6-9.7)	0.05
oxLDL(u/l)	56.4(29)	53.6(24)	0.5

Table 5.10: Indices of inflammation, oxidative stress, and thrombosis according tertiles of endothelial function amongst South Asians

	South Asians (n=229)		
	High	**Normal**	**P value**
hsCRP (mmol/l)	2.1(1.1-5.6)	1(0.8-1.8)	0.002
IL6 (pg/ml)	34.6(19-51)	29(19-46)	0.4
TNF (pg/ml)	132(92-188)	99(70-148)	0.01
VWF (u/dl)	112(102-135)	102(79-125)	0.01
E-Selectin (ng/ml)	25.8(15-40)	18(14-27)	0.008
HO (ng/ml)	9.3(7-11)	6.9(5.5-9.1)	0.003
oxLDL(u/l)	55(27)	52(20)	0.6

P value using Mann Whitney U test and independent t-test comparing poor vs. good tertiles, significance at <0.05 level

5.3.8 Analysis of indices of dyslipidaemia

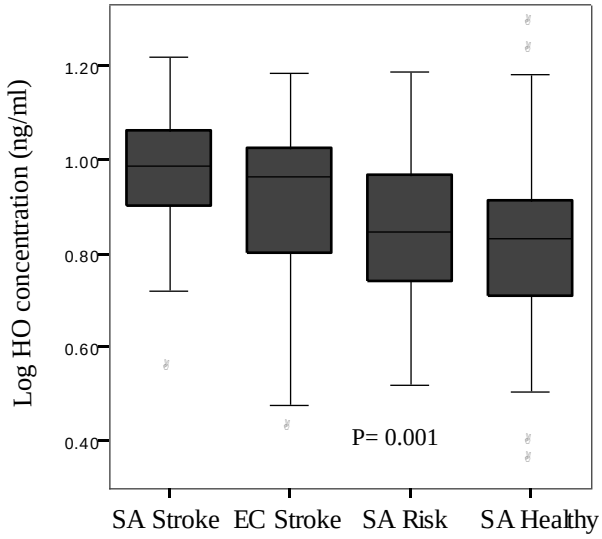
SA stroke patients had significantly lower Apo A and B levels compared to EC stroke patients (P<0.001) and SA controls (Table 5.8). Apo B/A levels were comparable across all groups. On univariate analysis Apo A levels significantly correlated with arterial stiffness (r= -0.32, P<0.001).

5.3.9 Analysis of indices of oxidative stress and thrombosis

SA strokes patients had significantly higher HO levels compared to both EC (P=0.02) and SA controls (P=0.001) (Figure 5.10). SA with hypertension (total) had significantly higher HO levels compared to people without hypertension (P<0.001), but this was not apparent in SA stroke patients. HO levels increased in an ordinal fashion across sub groups with and without hypertension (Pseudo R-Square=0.0.03, p=0.04) (Figure 5.11).

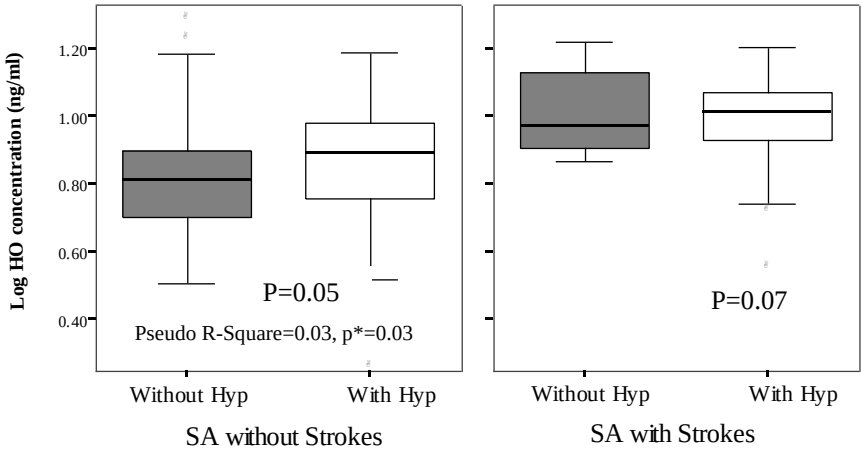
SA stroke patients had significantly higher oxLDL levels compared to EC stroke patients (P=0.01) and SA controls (P=0.001). oxLDL levels were similar across arterial stiffness and endothelial function groups in SA (Tables 5.10 and 5.11). SA stroke patients had significantly elevated E-Selectin levels compared to EC stroke patients (P<0.001) as well as SA controls (P<0.001). There was no significant difference in the levels of vWF between SA and EC. However, vWF levels were significantly raised amongst South Asians with poor endothelial function and arterial stiffness (Tables 5.10 and 5.11). In univariate analysis, HO level negatively correlated with endothelial function (r= -0.2, P=0.001) and associated with impaired endothelial function [Beta (95%CI): -2(-0.4—0.01). P=0.04], but after incorporating age, gender and other CVD risk factors into the model, this association became non-significant. In a separate analysis hypertension was significantly associated with HO level [Beta (95%CI):0.06(0.01-0.11), P=0.007]. On Receiver Operator Characteristic (ROC) curve analysis in the discrimination of subjects with poor endothelial function, HO was a better discriminator (AUC (95% CI):0.63(0.5-0.7), P=0.001) than IL6, TNF, vWF, HsCRP and E-Selectin levels.

Figure 5.10: Serum HO-1 concentration comparing South Asians vs. European Caucasians



P value using one way ANOVA comparing differences across all four groups,
significance at <0.05 level, SA:South Asian, EC:European Caucasian.

Figure 5.11: Serum Haemo Oxygenase concentrations amongst South Asians, in relation to stroke and hypertension status



P value using independent t-test comparing SA with and without hypertension,
p* using ordinal regression comparing increasing trend across four groups Significance at <0.05 level.

5.4 Discussion

This is the first study to comprehensively investigate the structural and functional properties of the arterial wall in a large population of migrant SA stroke survivors. In keeping with our first hypothesis, SA stroke survivors had significantly impaired endothelial function and increased arterial stiffness compared to age and gender matched EC stroke survivors. More importantly, of all the conventional risk factors, glycaemic status was independently associated with both arterial stiffness and endothelial function abnormalities. Given that this thesis work previously reported the higher prevalence of diabetes amongst SA stroke survivors compared to EC and impact of diabetes on SA stroke mortality (Chapter 3), where people with diabetes had poor five-year post-stroke survival rates, the present study provides a pathophysiological insight for these findings.

There was a significant interaction between diabetes status and South Asian ethnicity (Table 5.5). This supports the contention that diabetes status itself may explain, many of the vascular aberrancies observed amongst South Asian stroke survivors. Hence, not only diabetes is more common amongst SA compared to other ethnic groups (Chapter 3), but also a hereditable risk factor in this population (Moussouttas, et al., 2006). In the current study more than 40% of the SA stroke survivors had evidence of diabetes and more importantly patients with diabetes had the greatest vessel wall abnormalities. On the other hand, endothelial dysfunction independently associated with arterial stiffness. Thus, part of the remodeling processes that have occurred in the vessel wall could be attributed to a primary defect in the endothelium (Guerci, et al., 2001). Glycaemic status has an impact on the endothelium-derived Nitric Oxide (NO) production, causing a blunted smooth muscle relaxation response in the small to medium sized vessel walls with a consequent increase in vessel

tone, contributing to increased arterial stiffness (Cruickshank, et al., 2002). This hypothesis is further supported by our findings of the current analysis where, following the administration of GTN, both groups had similar reflective indices compared to those produced post-Salbutamol administration indicating similar dilation of the vessels wall in both ethnic groups in the presence of adequate NO availability. Nitric Oxide availability is increased using GTN rather than Salbutamol as the former is an external NO donor drug whilst the latter acts on the beta adrenoreceptors of the endothelium to release endogenous NO where the response is based merely on the functional integrity of the endothelium.

The second objective of my study was to investigate the impact of eNOS-NO axis indices on structural and functional properties of the arterial wall and pathogenic role of these determinants on cerebral haemodynamics and ischaemic strokes. In keeping with the second hypothesis, SA stroke survivors had significantly lower eNOS, NO and cGMP levels compared to EC and associated with higher arterial stiffness and poor endothelial function. Reasons for low levels of eNOS-NO axis parameters amongst South Asian stroke survivors in the current study are multi factorial. Possible explanations include down-regulation of the eNOS gene expression (Li, et al., 2001), dysfunction of eNOS enzymatic activity or heightened degradation of endothelial NO (Davignon and Ganz, 2004). The eNOS gene is regulated through multiple mechanisms and entails several polymorphisms, some of which bear functional consequences. Recently, many vascular genetic studies have demonstrated an association between certain eNOS gene polymorphisms (G894T, T-786C) and higher cardiovascular risk amongst certain ethnic groups (Mitchell, et al., 2007). Given the findings of the current study, it is likely that reduced eNOS levels are associated with an aberrant eNOS gene expression amongst the South Asian population. Conversely, other inflammatory processes, oxidative stress and

pro-thrombotic markers which are known to regulate eNOS gene expression (Yang, et al., 2006), and are thought be more prevalent amongst South Asian stroke survivors (Miller, et al., 2005; Kain, et al., 2005) may also have an adverse synchronised impact on the eNOS gene expression and consequent NO production.

In the current analysis, diabetes as a risk factor was significantly associated with reduced levels of eNOS-NO parameters. Although there are many possible mechanisms, the most likely explanation appears to be that, in the presence of diabetes, NADPH oxidase production in the vascular beds is increased, thereby enhancing the production of reactive oxygen species and related oxidative stress (Föstermann, et al., 2006). These factors are known to increase NO catabolism, impair cGMP activity and lead to consequent endothelial damage (Münzel, et al., 2005).

In keeping with previous studies (Cheng, et al., 2008), current analysis also demonstrated the putative link between the endothelium-derived Nitric oxide synthesis pathway and vessel wall characteristics. South Asian stroke survivors with very low levels of eNOS and NO were demonstrated to have higher arterial stiffness, indicating that part of the remodeling processes that has occurred in the vessel is attributed to a primary defect in the endothelium (Guerci, et al., 2001) (the inability to produce sufficient NO) causing a blunted smooth muscle relaxation response in the small to medium sized vessel walls with a consequent increase in vessel tone, contributing to increased arterial stiffness (Chowienczyk, et al., 1999). In addition, both NO as well as eNOS levels demonstrated the discriminatory capacity to identify people with increased arterial stiffness and poor endothelial function. This suggests that these markers may have an added utility in the identification of higher risk individuals (especially South Asians), enabling refinement of risk stratification in the community setting. This merits

further research. This part of the analysis may also provide a biochemical explanation (Calver, et al.,1992) for previous observations.

Systematical investigation of the influence of inflammatory, metabolic and oxidative stress related indices on structural and functional properties of the arterial wall was the third objective of this study. In keeping with our third hypothesis, SA stroke survivors had significantly deranged biochemical parameters coupled with abnormal vascular risk indices such as arterial stiffness and endothelial function.

South Asians with stroke were demonstrated to have increased inflammatory markers compared to those of EC as well as healthy controls. In particular, TNFα levels were significantly raised in South Asians irrespective of the risk factor burden or stroke status and coordinated with an increasing level of arterial stiffness and impaired endothelial function. As far as we are aware, this is the first study to report an association between increased TNF levels and arterial stiffness, although a small number of studies have shown improvement of vascular risk indices following anti TNF drug administration amongst people with rheumatoid arthritis (Komai, et al., 2008; Cypiene, et al., 2007). hsCRP level is the most validated sensitive clinical inflammatory marker in current practice and has been reported to be increased amongst healthy SA populations compared EC (Somani, et al., 2006). A small number of studies have also demonstrated an association between higher hsCRP levels and increased arterial stiffness, but in healthy populations who were not on any treatment (Duprez, et al., 2005; Kampus, et al., 2005). In the current study we did not observe any significant difference amongst SA stroke survivors compared to EC. It is likely that cardiovascular risk treatment may have an impact on the measurement of hsCRP levels, reducing the expected differences between the two ethnic groups. It is interesting to note that this unexpected finding of

comparable hsCRP levels but raised IL6 and TNF levels was also similarly reported in an ethnicity-based study investigating the differences between Afro-Caribbeans and EC healthy subjects (Kalra, et al., 2007).

In several recent clinical studies, the apolipoproteins levels, which reflect the cholesterol balance between potentially atherogenic and anti-atherogenic lipoprotein particles, have been reported to be better predictors of CV risk than any of the cholesterol indices (Walldius, G. and Jungner, 2006; Thompson, A. and Danesh, J., 2006). SA stroke survivors are known to have elevated Apo B to Apo A ratio compared to that of healthy SA (Sharobeem, et al., 2007), however its impact on vascular indices had not been previously elucidated. In the current analysis, SA stroke survivors with impaired endothelial function and higher arterial stiffness had significantly lower Apo A and B levels but comparable Apo B/A ratio compared to EC. It is possible that the impact of anti-cholesterol treatment may have influenced the true Apo A and B levels in both SA and EC populations but very unlikely to confound the comparative analysis on vascular indices, as both groups were on very similar treatment.

Haem-Oxygenase is a marker of oxidative stress and known to play significant role in the development role arthenogenesis (Kapturczak, et al., 2004) and cardiovascular disease (Duckers, et al., 2001). Increased levels of HO expression have been found in conditions associated with stroke (Chen-Roetling, et al., 2006), coronary artery disease (Chen, et al., 2008), diabetes (Adaikalakoteswari, et al., 2006) and hypertension (Botros, et al., 2005). However, available literature suggests that levels of HO expression may demonstrate different functional properties depending on the concentration and environment (Suttner, et al., 1999). For example increased HO levels in an atherosclerotic tissue or vascular bed indicate a protective role of HO (Juan, et al., 2001)

whereas in brain cells increased levels are associated with cell death (Chen-Roetling' et al., 2006). In the present analysis Heme oxygenase-1 (HO) levels were significantly elevated in SA stroke patients compared to that of controls as well as EC counterparts. More importantly, levels of HO were associated with impaired endothelial function. In sub group analysis, SA with hypertension had significantly higher HO levels compared to normortensives, and hypertensive status independently predicted higher HO levels. These findings are consistent with studies demonstrating raised levels of HO amongst hypertensives as a consequence of increasing blood pressure rather than the effect (Ishizaka, et al., 1996). In addition, SA stroke survivors also had increased oxLDL levels. Recent literature suggests that increased levels oxLDL found in atherosclerotic conditions have been known to promote HO expressions (Ishikawa' et al., 1996). Although this study is not designed to investigate these molecular interactions and vascular functions in different tissue beds, elevated levels of both HO and oxLDL amongst SA stroke survivors highly suggest increased evidence HO expression, as a result of cumulative oxidative stress status, amongst SA patients with strokes compared to that of EC counterparts.

E-Selectin, one of the studied markers of endothelial activation, was significantly elevated in SA stroke cohort, however it did not demonstrate any association with vessel wall characteristics. However, levels of E-Selectin levels had been reported to be comparable in healthy SA compared to that of EC, even though E-Selectin level in a higher risk SA population has not been previously investigated. In keeping with the previously published studies (Jaumdally, et al., 2007), levels of vWF did not demonstrate any ethnic differences. SA stroke patients with poor endothelial function however had significant higher vWF levels and, consistent with the current literature, indicating vWF as a marker of endothelial dysfunction (Blann, et al., 1993).

The present study also demonstrates the clear differences in the distribution of stroke subtypes, whereby SA stroke patients had more small vessel disease (lacunar infarctions) compared to more prevalent large vessel strokes amongst European Caucasians. This is consistent with other published studies (Syed, et al., 2003) particularly amongst a stroke population with higher prevalence of diabetes. However, De Silva et al (2007) recently demonstrated higher rates of intracranial large vessel disease even amongst patients with lacunar infarctions using intracranial doppler imaging. More studies using more sophisticated imaging methods are needed to determine exactly the type of intracranial vascular pathology amongst SA stroke patients.

Limitations: A potential limitation of this study is that this is not a true population-based study, as the majority of the patients were recruited from hospitals and the conclusions derived from this study may not be applicable for the total South Asian stroke populations. As previously reported (Chapter 1), the majority of the SA are known to seek hospital admission early (Hsu, et al., 1999) and no differences have been observed in hospital admission rates among SA when compared to other ethnic groups. However, assignment of ethnicity is not always reliable and the multiple methods used to identify SA ethnicity (self reported ethnicity, identification of South Asian names), minimises this potential source of bias. Socio-economic differences amongst two ethnic groups may have confounded the analysis including factors such as access to health care, but we attempted to minimise this effect by recruiting all the patients from areas with a similar deprivation index and from the same geographical region. Another potential limitation of this study was stroke sub-typing according to Bamford and TOAST classifications was performed by a stroke physician based on the available radiological and clinical information (CT reports, Duplex scan reports, and clinical details) at the time of the presentation. In our study, 12.3% of the SA and 6.7% of the EC had normal CT scans.

One of the reasons for apparently higher normal scan percentages in this study could be due very early imaging in some of these patients. In addition, CT scans of the smaller percentage of patients who had lacunar infarctions would have been reported as "normal" given that this study did not carry out any specialised imaging techniques such as MR /CT angiography, subtraction or perfusion-imaging techniques (Anderson, et al., 1994). There was also discrepancy in the percentages of larger and smaller vessel strokes between the two classifications. The reason for this could be multi-factorial, including observer and classification errors. An integral disagreement between these two classifications is known to exist and had also been reported in previous population based studies as well as randomized clinical trials (Anderson, et al., 1994; Lindenstrom, et al., 2001). The use of more sophisticated imaging techniques (MRI, MR angiography) may minimise these errors as previously mentioned. The possible classification errors due to bias were minimised by rechecking the stroke types against a standard questionnaire and reviewing the case notes accordingly by a second physician. The documented prevalence of cardiovascular risk profile (PVD) was based on the patient's past medical history and the available evidence from case notes. We didn't perform any diagnostic test such as ankle brachial index measurements. This may have underestimated the true prevalence rates in both ethnic groups.

Limitations of the current study include the use of single indirect indices to measure endothelial function and arterial stiffness. Four percent of the patients didn't manage to undergo complete vascular assessment protocol due to practical difficulties and demonstrate the limitation of using this technique in routine clinical practice. We advised patients to withhold most of the potent vasodilatory medications at least 48 hrs prior to the vascular measurements. However anti-platelet medication Dipyridamole, which is known to have a weak micro-vascular vasodilatory effect, was not asked to stop

due to ethical reasons and patients safety. This may have confounded the analysis. A study design which utilises combined methods of endothelial assessments (for example, FMD, Endothelial progenitor cells, von Willebrand factor analysis) would provide more comprehensive results.

In addition, some stroke patients had difficulties in standing up straight (<5%) and thus standing height measurements where not possible to be performed, so lying values or previous measurements documented on the case notes were used for the analysis. However, their weight was appropriately measured using a chair scale. The diagnosis of diabetes mellitus was also made on the basis of fasting blood glucose measurements and available documented evidence. None of the volunteers had an oral glucose tolerance test for specific disease exclusion

In conclusion, SA stroke survivors in the current hospital-based study have higher small vessel disease related cerebrovascular accidents compared to EC. Underlying glycaemic status in SA demonstrated to have a disproportionate impact on the vascular system, leading to aberrant remodelling of the vessel wall characteristics. South Asian stroke survivors were also found to have aberrances related to the endothelium-derived nitric oxide synthesis axis, thereby exerting a major impact on vessel wall characteristics. More research is warranted to further investigate the biochemical and genetic regulation of the eNOS-NO axis amongst South Asians.

In addition, the results of this study indicate that there are important ethnic differences in the markers of inflammation, oxidative stress and metabolic status, where clustering effects of these examined indices (Forouhi, et al., 2001) are likely to have an aberrant impact on the reported abnormal vessel wall characteristics amongst SA stroke survivors. Indeed, adverse diet and lifestyle differences observed in migrant SA may

further augment this unfavourable risk profile (Lean, et al., 2001). However, it appears that conventional risk factors, such as diabetes and hypertension, remain as the most detrimental factors in the aetiology of cerebrovascular disease in South Asians. The conclusions derived from this hospital-based study may not be generalisable to the total South Asian stroke population and would merit further investigation in a large population based study. [5]

[5]Gunarathne, A., Patel, J.V., Kausar, S., et al.(2009).Glycemic status underlies increased arterial stiffness and impaired endothelial function in migrant South Asian stroke survivors compared to European Caucasians: pathophysiological insights from the West Birmingham Stroke Project. **Stroke**, 40:2298-306

Chapter 6 : General Discussion

This thesis addressed several questions concerning the increased burden of cerebrovascular disease amongst the migrant South Asian population living in the United Kingdom using a number of cross-sectional, retrospective, prospective, case control and laboratory-based studies on stroke epidemiology and underlying pathophysiology.

6.1 Discussion Summary:

6.1.1 Prevalence of conventional cardiovascular risk profile in South Asians stroke survivors and impact on all cause mortality

Current literature appears to indicate that South Asians to have higher prevalence of diabetes and hypertension when compared to European Caucasians (Chapter 1). This was confirmed in the initial registry-based study, looking at a large South Asian stroke population. Further, this study provided insights into the changing CVD risk profile amongst South Asian stroke survivors over the last decade. The observed increased prevalence of both hypertension and diabetes might be explained by the fact that there has been improvement in the identification of risk factors among stroke patients. In Chapter 3, the magnitude and impact of risk from cardiovascular comorbidities on survival rates amongst South Asian stroke patients was investigated. Mortality following a stroke is governed by disease severity as well as other comorbidities, which have a direct impact on recovery rates. In this longitudinal analysis, 56% of the South Asian stroke patients were diabetic and cumulative survival at 5 years was markedly poorer in those persons with diabetes. This demonstrates the adverse impact of diabetes on long-term stroke outcomes amongst South Asians.

6.1.2 Indices of arterial stiffness in healthy South Asians compared to the general population

Literature review on arterial stiffness and ethnicity (Chapter 1.6) highlighted the significant paucity of available data on indices of arterial structure and function amongst healthy South Asians as well South Asians with established cardiovascular or cerebrovascular diseases.

In the first case control study, the principal objective was to determine the structural characteristics of the vessel wall amongst healthy SA compared to that of EC. It was hypothesised that there could be a markedly earlier progression of disease in South Asians, indicating that the pathogenesis of sub-clinical atherogenesis and premature arteriosclerosis could become manifest even in apparently healthy individuals. My data show that apparently healthy South Asians to have higher arterial stiffness compared to that of age- and gender-matched European Caucasians. More importantly, mean arterial pressure was a significant independent predictor of arterial stiffness. In an exploratory analysis, South Asians were demonstrated to have a greater arterial stiffness for a given blood pressure category after adjusting for other comorbidities including age. Some of the possible reasons for this paradox of higher arterial stiffness despite lower mean arterial pressure levels in South Asians have been discussed in detail in Chapter 4 and merits further investigations.

6.1.3 Stroke characteristics amongst South Asian stroke survivors

Very few previous studies have reported data on stroke subtypes in south Asians. In the current study, an attempt was made to classify strokes according Bamford and TOAST classifications. The present hospital-based study demonstrates a clear difference in the distribution of stroke subtypes, whereby SA stroke patients had more small vessel disease (lacunar infarctions) compared to more prevalent large vessel strokes amongst

European Caucasians. This is consistent with other published studies and given higher prevalence of diabetes amongst South Asians. However, as previously highlighted, the used classification methods have potential limitations.

6.1.4 Indices of arterial stiffness and endothelial dysfunction in South Asian stroke survivors

The pathophysiology of excess cerebrovascular disease mortality amongst South Asian stroke survivors had not been previously investigated. Therefore the objective of the second case control study was to examine the structural and functional vessel wall characteristics amongst the higher CVD risk South Asian stroke population. My data demonstrate that SA stroke survivors to have significantly impaired endothelial function and increased arterial stiffness compared to an age and gender matched EC population, results which, to the author's knowledge, have not previously been replicated in other studies. More importantly, of all the conventional risk factors, glycaemic status independently associated with both structural and functional vascular abnormalities.

Given the higher prevalence of diabetes amongst SA stroke survivors compared to EC and impact of diabetes on SA stroke mortality (Chapter 3) where people with diabetes had poor five year post-stroke survival rates, in this chapter we were able to provide possible pathophysiological explanations for findings, that the authors have previously reported. According to the interaction analysis of South Asian ethnicity and Diabetes status on vascular parameters, Diabetes status and SA ethnicity demonstrated a significant interaction in determining increased arterial stiffness but not with impaired endothelial function. Reason for this observation could be multi-factorial, including underlying aberrant genetic susceptibility and other socio-economic factors. This study also indicated that part of the remodeling processes that have occurred in the vessel wall

could be attributed in part to a primary defect in the endothelium and consequent but related Nitric Oxide availability (eNOS – NO axis).

6.1.5 Possible biochemical basis for abnormal vessel wall characteristics amongst South Asian stroke survivors

The biochemical basis for abnormal vessel wall characteristics amongst South Asians had not been previously investigated. Recognising the results of the previous study (Chapter 5), I specifically investigated the cascade of endothelially-derived nitric oxide production and its relation to the structural and functional properties of the vessel wall, a relationship which may increases the susceptibility to ischaemic strokes amongst South Asians. In keeping with the original hypothesis, SA stroke survivors had significantly lower eNOS, NO and cGMP levels compared to EC, coupled with an increasing gradient of higher arterial stiffness and poor endothelial function. More importantly, diabetes was significantly associated with reduced levels of eNOS-NO parameters.

Given the higher prevalence of diabetes (Chapter 3) amongst SA stroke survivors compared to EC and the impact of diabetes on increased arterial stiffness (Chapter 5) and SA stroke mortality (Chapter 3), the results of this study further emphasize the importance of diabetes as perhaps the single most important risk factor amongst South Asians and this may provide some pathophysiological and biochemical explanation for previous observations. Possible reasons for low levels of eNOS-NO axis parameters in this study amongst South Asian stroke survivors have previously been discussed (Chapter 5.3.6) and merit further research. The analysis of data from this sub study highlights the importance of further investigating the reasons for impaired eNOS activity and reduced NO availability, in particular at the examination of degradation of

endothelial NO as a result of adverse metabolic, inflammatory or oxidative stress related mechanisms.

6.1.6 Impact of inflammatory, metabolic and oxidative stress related indices on structural and functional properties of the arterial wall in South Asian stroke survivors.

Building on the results of the previous studies, a cross-sectional study was carried out to systematically investigate the influence of inflammatory, metabolic and oxidative stress related indices on structural and functional properties of the arterial wall in the migrant SA stroke population. This study suggests that the combined effects of metabolic, inflammatory and oxidative stress related abnormalities may contribute to the reported (Chapter 5.3.7) abnormal vessel wall characteristics amongst SA stroke survivors. However it is likely that these factors may have an adverse impact on the vessel wall properties directly or cumulatively with the already established conventional risk factor burden.

In this study, Haem-Oxygenase was used as a marker of oxidative stress, which is also known to play significant role in the development of atherogenesis and cardiovascular disease. In my analysis, HO levels were significantly higher in SA with hypertension compared to normortensives. These findings have important implications for the SA stroke population (Chapter 5.3.9), again highlighting the importance of blood pressure regulation in CVD risk reduction. In this study, I concluded that the clustering effects of the metabolic, inflammatory and oxidative stress-related abnormalities may contribute to the reported abnormal vessel wall characteristics amongst SA stroke survivors. Conventional risk factors such as diabetes and hypertension, which are more prevalent amongst SA still remain as the most detrimental factors in the aetiology of cerebrovascular disease.

6.1.7 The utility of novel non-invasive vascular indices for early identification of high risk populations

As previously highlighted, South Asians, both with or without established risk factors, have a disproportionately higher cardiovascular risk compared to that of European Caucasians. However, currently available risk estimation methods are known to underestimate the actual risk within high risk populations. More importantly, the majority of available methods have proved to be both technically difficult to perform and time consuming, specifically amongst large populations and in community settings, factors that led to the two cardiovascular risk stratification sub studies within this thesis work. The aim was to assess the usefulness of non-invasive imaging technique (digital pulse wave form analysis) to examine the underlying pathophysiological processes for early identification of high risk populations. My data (2.6.3) appear to demonstrate the added utility of the SI and RI in identifying higher risk groups compared to the conventional cardiovascular risk indices. More importantly, this study highlights the importance of the use of novel risk indices to identify apparently healthy, but with underlying higher CHD/CVD risk, populations who may be missed in traditional risk assessment processes

6.2 Limitations Summary:

Limitations, which are specific to each sub study, have been previously discussed. Please see sections 2.6.3.5, 3.4.1, 4.4, and 5.4 for details. In summary, a major limitation of this thesis is that it is not a true population-based study, and the conclusions derived may not be applicable for the total South Asian stroke population. Development of a stroke registry based on ICD coding and hospital stroke admissions may have caused a selection bias and under-estimation of stroke rates. However, it is unlikely these errors had a significant impact on the comparative analysis, as they would have equally affected

both ethnic groups. Another potential limitation of this study is that stroke sub-typing according to Bamford and TOAST classifications was performed purely based on available CT and clinical information and may not have the power to identify vascular characteristic in stroke subtypes. The use of sophisticated imaging techniques may have aided more accurate stroke subtype classification. In addition, the use of indirect indices to measure endothelial function and arterial stiffness within this thesis is a potential limitation, but these methods have been validated previously and used in many other clinical studies. The combined use of other complex vascular assessment methods such as PWV and FMD may have given a comprehensive and more accurate assessment of the vascular structure and function (Chapter 5.4). I performed many subgroup analyses in addition to the original hypothesis testing and this may have caused "multiple comparisons". I have not used any specific statistical test such as Bonferroni correction to counteract and minimise this effect. This is a potential limitation of the study analyses.

6.3 Key findings and Conclusions

6.3.1 Epidemiological data available on South Asian stroke survivors remain limited and the interpretation and appraisal of available studies is restricted by methodological weaknesses such as cross-sectional design, small sample sizes, poor case ascertainment methods and recording, publication biases.

6.3.2 My data indicate that the prevalence of diabetes and hypertension are higher amongst South Asians stroke patients and there has been a reduced decline in stroke admissions in South Asians compared to European counterparts. (Chapter 3) These observed ethnicity related disparities warrant further investigation

6.3.3 Analysis of registry data (Chapter 3) show that five year all-cause mortality in South Asians with ischaemic stroke is associated with presence of diabetes mellitus and highlights the possible benefits of early and intensive CVD risk modification strategies amongst South Asian stroke survivors.

6.3.4 A key finding from this hospital-based study was that South Asian stroke survivors (when compared to EC) have a higher prevalence of small vessel disease strokes, which appears to be related to underlying glycaemic status (Chapter 5). Glycaemic status appears to exert a disproportionately negative impact on the vascular system, leading to aberrant vessel wall characteristics. There was however a significant interaction between diabetes status and South Asian ethnicity. This supports the contention that diabetes status itself may explain many of the vascular aberrancies observed amongst South Asian stroke survivors.

6.3.5 My data show that South Asian stroke survivors to have abnormalities related to the endothelium-derived nitric oxide synthesis axis, exerting a major impact on vessel wall characteristics (Chapter 5). In addition there were important ethnic differences in the markers of inflammation, oxidative stress and metabolic status. However only HO, TNF alpha and Apo A levels had significant independent associations with vessel wall characteristics amongst South Asians. In particular, HO was a better indicator discriminating South Asian stroke patients with poor endothelial function (when compared to all other markers studied) indicating its potential usefulness as a basis for finer CVD risk stratification.

6.3.6 Assessment of the vascular parameters in an apparently healthy South Asian population demonstrated, South Asians to have increased systemic arterial stiffness compared to European Caucasians (Chapter 4). There was an adverse and

disproportional impact of age and mean arterial pressure on the vascular system in South Asians. Pathophysiological differences in vessel wall characteristics even amongst healthy South Asians may explain their increased susceptibility to vascular disease including strokes.

6.3.7 My data appear to indicate the added utility of Indices of arterial stiffness (SI) for better CVD risk stratification (Chapter 2.6.3). In addition, DVP analysis technique also appears to provide a non-invasive method of measuring vessel wall characteristics in clinical practice.

In conclusion, of all the studied risk factors, diabetes appears to be the most detrimental modifiable risk factor in the aetiology of cerebrovascular disease amongst South Asians and highlights the benefits of early intensive CVD risk modification strategies amongst South Asian stroke survivors with diabetes.

6.4 Recommendation for future research

6.4.1 As acknowledged above, the conclusions derived from this thesis are based on hospital-based studies and may not be directly generalisable to the total South Asian stroke population. Therefore, the important findings of this thesis need to be repeated in a large population-based study, the design of which is also capable of examining the ethnicity related socio-economic, environmental and genetic factors.

6.4.2 It was repeatedly demonstrated that diabetes is a detrimental risk factor in the South Asian stroke population. However, it remains unclear exactly how diabetes contributes to abnormal vessel wall characteristics leading to higher all-cause mortality. Possible explanations such as underlying aberrant molecular mechanisms and genetic

polymorphisms influencing higher arterial stiffness and impaired endothelial function amongst South Asians need to be investigated.

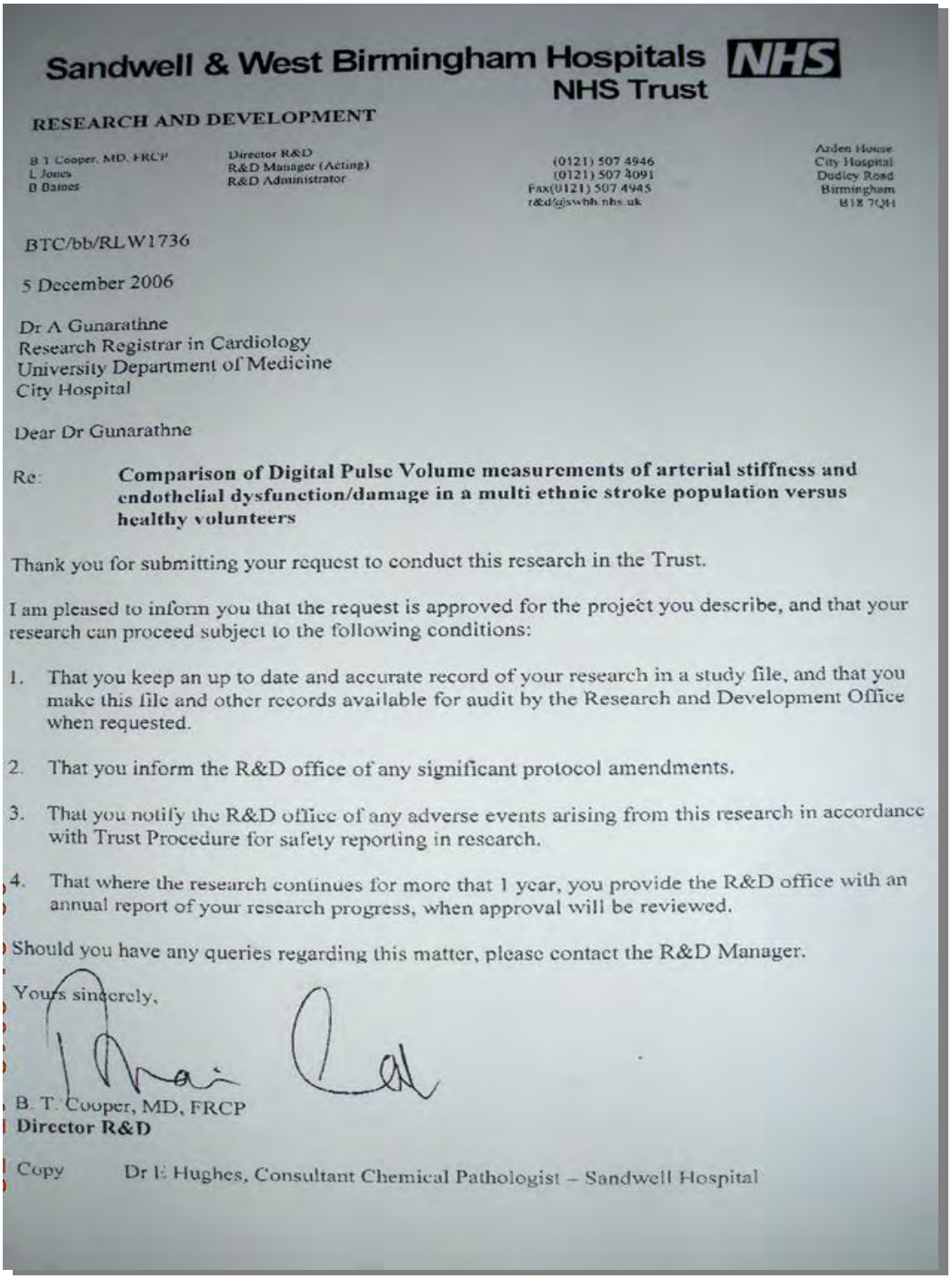
6.4.3 In the current study, stroke sub-classification was performed according the Bamford and TOAST classifications based on the CT imaging and available clinical data. However future studies using more sophisticated imaging methods (Diffusion weighted CT/MRI, intracranial Doppler imaging and CT angiography) are needed to determine more exactly the type of intracranial vascular pathology amongst SA stroke patients.

6.4.4 South Asian stroke survivors had significantly lower eNOS and NO levels positively correlated with an increasing gradient of higher arterial stiffness and poor endothelial function. Given that genetic studies on vascular stiffness have recently indicated an association between certain eNOS gene polymorphisms (G894T, T-786C) and higher cardiovascular risk amongst certain ethnic groups, expression of the eNOS gene amongst members of the South Asian population should be investigated.

6.4.5 Whilst indices of arterial stiffness (SI) measured using the DVP technique appeared to demonstrate a discriminatory usefulness in identifying members of high risk populations, there is a need for further studies to assess the external validity of the stiffness index and its ability to prospectively predict clinical outcomes to a greater degree than existing CVD risk score estimations. Further studies are also needed to assess the usefulness of arterial stiffness to monitor treatment efficacy and disease progression as well as ease of use and cost effectiveness.

Appendices

Appendix 1: Ethics Approval from the Sandwell & West Birmingham ethics committee.



Sandwell & West Birmingham Hospitals NHS Trust

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5 December 2006

Dr A Gunarathne
Research Registrar in Cardiology
University Department of Medicine
City Hospital

Dear Dr Gunarathne

Re: **Comparison of Digital Pulse Volume measurements of arterial stiffness and endothelial dysfunction/damage in a multi ethnic stroke population versus healthy volunteers**

Thank you for submitting your request to conduct this research in the Trust.

I am pleased to inform you that the request is approved for the project you describe, and that your research can proceed subject to the following conditions:

1. That you keep an up to date and accurate record of your research in a study file, and that you make this file and other records available for audit by the Research and Development Office when requested.
2. That you inform the R&D office of any significant protocol amendments.
3. That you notify the R&D office of any adverse events arising from this research in accordance with Trust Procedure for safety reporting in research.
4. That where the research continues for more that 1 year, you provide the R&D office with an annual report of your research progress, when approval will be reviewed.

Should you have any queries regarding this matter, please contact the R&D Manager.

Yours sincerely,

B. T. Cooper, MD, FRCP
Director R&D

Copy Dr E Hughes, Consultant Chemical Pathologist – Sandwell Hospital

Appendix 2: von Willebrand Factor assay procedure

- Microtitre plate coated overnight at 4^{o}C with primary antibody (Rabbit anti-human vWf polyclonal antiserum, DakoCytomation, UK, 35µl diluted in 20ml coating buffer, 100µl per well).Wash plate x3
- Samples (citrated plasma), and standards with known concentration of vWF maintained within the laboratory (250IU/dl, 150IU/dl, 100IU/dl (WHO standard), 50IU/dL) defrosted. Samples diluted 1/40 with PBS tween. 100µl of standards and samples added to wells in duplicate and incubated for 1 hour at room temperature. Wash plate x3
- Conjugation with secondary antibody (Rabbit anti-human vWf polyclonal antiserum conjugated to horse radish peroxidase, DakoCytomation, UK, 35ul diluted in 20ml coating buffer, 100µl per well) incubated for 45 minutes at room temperature. Wash plate x3
- Substrate produced by mixing 20ml Citrate Phosphate buffer with one 10mg ortho-phenylamine diamine tablet and 10µl hydrogen peroxide. 100µl of substrate added to each well.
- Colour development stopped after approximately 30 seconds with 100µl/well stop solution.
- Read with ELISA plate reader set to 492nm.

Appendix 3: E-Selectin assay procedure

- Microtitre plate coated overnight at 4^{o}C with primary antibody (Sheep anti-human E-Selectin polyclonal antiserum, R&D systems, UK, diluted to 1µg/ml in coating buffer, 100µl per well). Wash plate x3
- Samples (citrated plasma), and recombinant standard (recombinant 59kDA portion of the extracellular domain of E-Selectin, R&D systems) defrosted. Standard diluted to 50ng/ml and 200µl added to the plate in duplicate, then seven serial 2/1 dilutions carried out on the plate. Samples diluted 1/5 with PBS tween. 100µl samples added to wells in duplicate and incubated for 2 hours at room temperature. Wash plate x3
- Addition of secondary antibody (Biotinylated Mouse monoclonal anti-human E-selectin, R&D systems, diluted to 250ng/ml in PBS tween, 100ul per well) incubated for 2 hours at room temperature. Wash plate x3
- Conjugation with streptavidin-horseradish peroxidase (R&D systems) diluted 1/100 with PBS tween, 100µl per well, incubate for 20 minutes at room temperature in the dark. *NB. streptavidin binds avidly to biotin on the secondary antibody* Wash plate x3
- Substrate produced by mixing equal volumes of Substrate reagent A (stabilised peroxidase solution) and B (Stablised chromogen solution) – R&D systems - and immediately adding 100µl to each well. Colour development stopped after 3-5 minutes with 100µl/well stop solution.
- Read with ELISA plate reader set to 450nm.

Appendix 4: TNF assay procedure

- Microtitre plate coated overnight at 4^{o}C with primary antibody (Mouse anti-human TNFα, (Duo Set – R&D systems), diluted to 4.0μg/ml with PBS, 100ul per well). Wash plate x3
- Blocking Step: Block with 5% Marvel milk substitute (500mg per 10ml PBS) 200μl per well, 2 hours at room temperature. Wash plate x3
- Samples (EDTA plasma) defrosted. Standard (Duo Set, R&D systems) diluted to 1000pg/ml and 200μl added to the plate in duplicate, then six serial 2/1 dilutions carried out on the plate. Samples diluted to 1/5 with PBS Tween, 100μl added to wells in duplicate and incubated for 2 hours at room temperature. Wash plate x3
- Addition of secondary antibody (Biotinylated goat anti-human TNFα, Duo Set, R&D systems, diluted to 75ng/ml with PBS tween, 100μl per well) incubated for 2 hours at room temperature. Wash plate x3
- Conjugation with streptavidin-horseradish peroxidase (R&D systems) diluted 1/100 with PBS tween, 100μl per well, incubate for 20 minutes at room temperature in the dark. *NB. Streptavidin binds avidly to biotin on the secondary antibody.* Wash plate x3
- Substrate produced by mixing equal volumes of Substrate reagent A (stabilised peroxidase solution) and B (Stablised chromogen solution) – R&D systems - and immediately adding 100μl to each well.
- Colour development stopped after maximum 5 minutes with 50μl/well stop solution.
- Read with ELISA plate reader set to 450nm. Any samples below detection limit

  repeated using neat plasma instead of 1/5 dilution.

Appendix 5: Interleukin 6 assay procedure

- Microtitre plate coated overnight at 4^{o}C with primary antibody (monoclonal mouse anti-human IL-6 antibody R&D systems, UK, diluted to 4µg/ml with coating buffer, 100µl per well). Wash plate x3
- Blocking Step: Block with 4% Marvel milk substitute (400mg per 10ml PBS) 100µl per well, 1½ hours at room temperature Wash plate x3
- Samples (citrated plasma) and standard (R&D systems) defrosted. Standard diluted to 1250pg/ml and 200ul added to the plate in duplicate, then nine serial 2/1 dilutions carried out on the plate. Samples undiluted. 100µl samples added to wells in duplicate and incubated for 2 hours at room temperature. Wash plate x3
- Addition of secondary antibody (Biotinylated goat anti-human IL-6, R&D systems, diluted to 50ng/ml with PBS tween, 100µl per well) incubated for 1½ hours at room temperature. Wash plate x3
- Conjugation with streptavidin-horseradish peroxidase (R&D systems) diluted 1/100 with PBS tween, 100µl per well, incubate for 20 minutes at room temperature in the dark. *NB. streptavidin binds avidly to biotin on the secondary antibody*
- Substrate produced by mixing equal volumes of Substrate reagent A (stabilised peroxidase solution) and B (Stablised chromogen solution) – R&D systems - and immediately adding 100µl to each well.Colour development stopped after 30 minutes with 100µl/well stop solution.
- Read with ELISA plate reader set to 450nm.

Appendix 6: Nitrate assay procedure

- **Sample preparation**

All samples were diluted 2-fold using Reaction Buffer (1X). (100 ml sample + 100 ml Reaction Buffer (1X).

- **Reagent preparation**

All reagents were brought (except Nitrate Reductase) to room temperature before use. Distilled water was used when reconstituting to avoid nitrite/nitrate contamination.

- **Used Reagents:**

o **Microplates** (Part R80-0144) - Two 96 well microplates with removable strips.
o **Nitrate Reductase** (Part R80-1347) - 1 vial of lyophilized Nitrate Reductase, desiccated.
o **Nitrate Reductase Storage Buffer** (Part R80-0255) - 4 mL of a buffer containing preservatives.
o **NADH** (Part R80256 - inner vial; Part R80-0258 - bottle) - 2 vials of lyophilized reduced -Nicotinamide adenine dinucleotide, desiccated.
o **Nitrite Standard** (Part R80-0224) - 0.5 mL of a Sodium Nitrite solution (2000 _mol/L) with preservative.
o **Nitrate Standard** (Part R80-0223) - 0.5 mL of a Sodium Nitrate solution (1000 _mol/L) with preservative.
o **Reaction Buffer Concentrate (10X)** (Part R80-0257) - 30 mL of a 10-fold concentrated buffer containing detergent and preservative.
o **Griess Reagent I** (Part R80-0227) - 12 mL of Sulfanilamide in 2 N HCl.
o **Griess Reagent II** (Part R80-0228) - 12 mL of N-(1-Naphthyl) ethylenediamine in 2 N HCl.

- ***NADH Reagent***

1. Reconstitution – NADH was reconstituted with 1.0 mL distilled water. NADH was allowed to sit for 3 minutes with gentle agitation prior to use and was kept on ice for the duration of the assay.
2. Dilution – 900ml of NADH was diluted with 1.8 mL of distilled water and kept on ice for the duration of the assay.

- ***Nitrate Reductase***

1. Reconstitution - Nitrate Reductase was reconstituted with 1 mL Nitrate Reductase Storage Buffer. It was vortexed vigorously and was allowed to sit for 15 minutes at room temperature. 2. Dilution - Nitrate Reductase was diluted with 10ml of the reaction buffer and was placed on ice and was used within 15 minutes of dilution.

- **Assay steps**

1. All reagents were prepared and samples as described previously
2. 200 ml of Reaction Buffer (1X) was added to the Blank wells.
3. 50 ml of Reaction Buffer (1X) was added to the zero standard wells.
5. 50 ml of Nitrate Standard or sample was to the remaining wells.
6. 25 ml of NADH was added into all standard and sample wells.
7. 25 ml of Nitrate Reductase was added into all standard and sample wells and was mixed well and cover with the provided adhesive strip.8. Incubated for 30 minutes at 37° C.9. 50 ml of Griess Reagent I was added to all wells except Blank wells. 10.50 ml of Griess Reagent II was added to all wells except Blank wells. Mixed well by tapping the side of the plate gently.11. Incubated for 10 minutes at room temperature.12. Optical density (OD) was determined using a microplate reader set at540 nm.

Appendix 7: eNOS assay procedure

- **Used Reagents:**
  - **eNOS Microplate** (Part 890702) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against eNOS.
  - **eNOS Conjugate** (Part 890703) - 21 mL of polyclonal antibody against eNOS conjugated to horseradish peroxidase, with preservatives.
  - **eNOS Standard** (Part 890704) - 40 ng of recombinant human eNOS in a buffered protein base with preservatives, lyophilized.
  - **Calibrator Diluent RD5K** (Part 895119) - 21 mL of a buffered protein base with preservatives.
  - **Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.
  - **Colour Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.
  - **Colour Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).
  - **Stop Solution** (Part 895032) - 6 mL of 2 N sulphuric acid.
- **Reagent preparation**

All the reagents were brought to room temperature before use.

Wash Buffer – Was warmed to room temperature and was mixed gently until the crystals have completely dissolved. 20 mL Buffer was diluted with 480 ml of distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Colour Reagents A and B was mixed together in equal volumes To be used within 15 minutes

eNOS Standard – 1ml of eNOS standard was reconstituted with 1 mL of distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Standard was allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.900 µL of Calibrator was pipetted to the diluent RD5K (4000 pg/mL tube). 500 µL of the Calibrator was Pipetted into the remaining tubes. Stock solution was used to produce a dilution series. The 4000 pg/mL standard used as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).

- **Assay procedure:**

1. all the reagents, working standards and samples were prepared
2. 100 µL of Assay Diluent RD1W was added to each well.
3. 100 µL of standard, control, and sample was added per well and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
4. Each well was aspirated and washed repeatedly three times by filling each well with Wash Buffer (400 µL) using a squirt bottle, multi-channel pipette. After the last wash, any remaining was removed by decanting. Plate was inverted and blotted against clean paper towels.
5. 200 µL of eNOS conjugate was added to each well. And kept for 2 hours at room temperature on the shaker.
6. aspiration/wash step was repeated as in step 4.
7. 200 µL of Substrate Solution was added to each well and incubated for 30 minutes at room temperature.50 µL of Stop Solution was added to each well.
8. Optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

Appendix 8: cGMP assay procedure

- **Used Reagents:**
  - **Goat Anti-rabbit Microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with a goat anti-rabbit polyclonal antibody.
  - **cGMP Conjugate** - 6 mL/vial of cGMP conjugated to horseradish peroxidase with preservatives and red dye.
  - **cGMP Standard** - 5000 pmol/vial of cGMP in buffer with preservatives, lyophilized.
  - **Primary Antibody Solution** - 6 mL/vial of rabbit polyclonal antibody to cGMP in buffer with blue dye and preservatives.
  - **Calibrator Diluent RD5-5** - 21 mL/vial of a buffered protein solution with preservatives.
  - **Wash Buffer Concentrate** - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.
  - **Colour Reagent A** - 12.5 mL/vial of stabilized hydrogen peroxide.
  - **Colour Reagent B** - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).
  - **Stop Solution** - 6 mL/vial of 2 N sulphuric acid.
- **Reagent preparation**
  - **Substrate Solution** - Colour Reagents A and B was mixed together in equal volumes to be used within 15 minutes of use.
  - **cGMP Standard** - The cGMP Standard was reconstituted with 1.0 mL of distilled water. This reconstitution produces a stock solution of 5000 pmol/mL. standard was mixed to ensure complete reconstitution and allowed to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. The 500 pmol/mL standard was used as the high standard and Calibrator Diluent RD5-5 or served as the zero standard
- **Assay procedure:**

1. All the reagents, working standards and samples were prepared as directed in the previous sections.
2. 150ml of the appropriate diluent was added to the NSB wells and 100 ml to the zero standard (B0) wells. Calibrator Diluent RD5-5 used
3. 100 ml of Standard, control, sample was added to the remaining wells.
4. 50ml of cGMP conjugate was added to each well. Colour changed to red colour.
5. 50ml of the Primary Antibody Solution was added to each well (excluding the NSB wells). All wells except the NSB wells became violet in colour.
6. Incubated for 3 hours at room temperature on a horizontal orbital microplate shaker 500 rpm.
7. Each well was aspirated and washed (4x) using the wash Buffer
8. After the last wash, any remaining was removed by decanting. Plate was inverted and blotted against clean paper towels.200 ml of Substrate Solution was added to each well and Incubated for 30 minutes at room temperature on the bench top. 50 ml of Stop Solution was added to each well. The colour in the wells changed from blue to yellow. Optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm.

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