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Table of Contents

Emerging infectious diseases and the effect of climate change

James Farrugia, Yanika Farrugia, Chantal Vella,
Charles Mallia Azzopardi

Infectious diseases have been around since the dawn of time, having afflicted human civilizations for millennia.[1] In 2007, WHO warned that since 1970, infectious diseases have been emerging at a rate never seen before.[2] These emerging health threats come with significant socio-economic costs and both direct and indirect impacts on healthcare systems, disrupting economic activity.[3]

The exact nature as to how and why these infections are changing is multifaceted and involves the interplay of many, frequently poorly determinable factors. With the rising human population, people living in closer quarters and travelling wider and more frequently than ever before, the potential for epidemics is high.[4] With ongoing climate change and rampant ecological degradation, further epidemics seem inevitable. In this article I will be focusing on the effect of global climate change on emerging infections.

James Farrugia MD, MRCP (Uk)*
Department of Medicine,
Mater Dei Hospital
Msida, Malta
james.a.farrugia@gov.mt

Yanika Farrugia MD, MRCP (Uk)
Department of Medicine,
Mater Dei Hospital
Msida, Malta

Chantal Vella MD, MRCP (Uk)
Department of Medicine,
Mater Dei Hospital
Msida, Malta

Charles Mallia Azzopardi MD, FRCP, DTM&H
Department of Medicine,
Mater Dei Hospital
Msida, Malta

**Corresponding Author*

How do we define emerging and re-emerging infections?

The term 'Emerging infections' is broad and may be subcategorized into (i) previously undetected infections, (ii) known diseases which have spread geographically, (iii) infectious diseases with potential for bioterrorism, and (iv) those infections which had diminished in importance or frequency in the past, but are now resurfacing either due to a change in environment, society or virulence, or due to increasing antimicrobial resistance. The latter may be referred to as 're-emerging infectious diseases'.[4]

What about the effects of climate change?

Global warming, nowadays preferentially known as 'climate change', is one of the most complex challenges of the century. It is not yet clear whether the recent global temperature increases are solely attributable to human enterprise or whether they are also a function of a cyclical variation.[5] What is undeniable is that global temperatures have risen significantly since 1900 and during this century they will likely exceed the 'safe' 2°C threshold above average.[6] Furthermore, the average sea level has been rising by 1.8mm per year since 1961 and Arctic ice has been shrinking by 2.7% per decade.[7] Controversy still shrouds predictions of the consequences of climate change on infectious diseases, despite several climate-based models. Predictions range from descriptions of a worldwide spread of some infections, to more conservative analyses showing diseases expanding into areas while vanishing from others.[8] The latter scenario seems more likely, as most species, including infectious diseases and their vectors, have both upper and lower thresholds to temperature tolerance.[9]

So how does climate change affect infectious diseases?

Vector-borne Infections

Many infectious diseases are dependent on vectors for their transmission to humans. Mosquitos, ticks, and fleas are directly affected by changes in temperature, rainfall[10] and humidity.[7] For example, aquatic larvae of mosquitoes require pools of water for maturation, with several studies showing a positive correlation between heavy rainfall and outbreaks of mosquito-borne illnesses,[11] while flea diversity declines with increasing altitude and latitude.[12] Climatic changes may thus cause a progressive geographical shift of disease vectors from their endemic areas to locations where they were traditionally categorized as rare.[10] Rising temperature also affects the insect density within an area, the vector's rate of reproduction, the rate of pathogen maturation and replication within the vector itself, while increasing insect bite frequency.[13] In such a case, malaria, dengue fever and tick-borne encephalitis to name a few, may spread or translocate to different regions, potentially interfacing with human populations having little or no immunity to these diseases.[6] Climate change is less likely to result in new infectious diseases and more likely to result in a change in disease distribution, with the areas at highest risk being marginal areas where vectors can survive.[14]

Water-borne Infections

Water borne infections may also be affected by climate change. During times of drought, poor sanitation may result from water scarcity, with the population exposed to potentially contaminated water. Increased infection may also occur during times of flooding, from overwhelmed sewage lines or runoff from livestock excrement.[15] For example, cryptosporidiosis is related to severe weather events, and overwhelmed sewage treatment plants.[16] Flood-associated reports of hantavirus pulmonary syndrome from deer mice foraging in homes and outbreaks of leptospirosis were reported in South America and South-East Asia.[17] Climate related increases in sea temperature and level may also lead to a higher incidence of waterborne and toxin-related illnesses such as cholera and seafood poisoning.[10] In fact, Vibrio spp. bacteria native to the North Sea and the Baltic region, were shown to grow faster in a warmer summer in 2006,[18] while replication of Salmonella increases as temperature rises to 37°C.[7]

Migration

Since as many as 75% of emerging infectious diseases are zoonoses,[19] both human and animal migration play an important role in disease transmission. Human migration is not a new phenomenon - a search for better prospects, escaping civil strife and religious or political persecution are few of the many reasons for migration. Forecasts for climate change-induced migration vary from 25 million to 1 billion people by 2050, with 200 million being most widely quoted figure.[20] The proposed mechanisms for this migration include (i) the increase in severity and frequency of extreme climatic events, (ii) the potential loss of arable and habitable land due to sea-level rise and (iii) negative impacts on ecosystems which sustain livelihood, all of which would promote relocation to greener pastures.[21] Migrants may be exposed to new infections along their travel - to diseases which they might not have sufficient immunity or socio-cultural experience for. For example, people moving to areas with high malaria prevalence would be at particular risk of infection, morbidity, and mortality. Migrants may also act as carriers of infection along their journeys, exposing non-immune natives and introducing infections which may have previously been rare to the area.[21] The ongoing epidemic of cutaneous leishmaniasis in Syrian refugees in Lebanon, a country in which this disease had been previously hypoendemic is an example of this.[22] Sexually transmitted infections, Hepatitis B, HIV and Tuberculosis may also be spread via this route. Moreover, migrants might re-introduce infectious agents upon return to their native country, having been exposed during their travels.[21]

Airborne

Climate influences the pathogenesis of airborne infectious diseases through temperature, level of humidity and wind. Most notably, the influenza virus is reported to survive better at cold temperature and low relative humidity. A low relative humidity decreases mucosal barriers against infection, allows for better evaporation of bioaerosols from infected mammals and maintains viral particle stability in the air. Mammals were also found to shed viral particles in higher

quantities at lower temperature, although their innate immunity was not weakened by this.[23] It has also been suggested that wind may help transmission of viruses by transporting them across oceans on dust particles.[7]

Conclusion

Predictions of tropical diseases moving into wealthier, temperate regions have caught the public's attention.[9] While some regions may see reductions in infectious disease, populations in areas where infections will expand have genuine cause for concern.

Despite our incomplete knowledge of the full impact of climate change on infectious diseases, we should not remain passive bystanders to this. Countries should be proactive in taking measures based on regional scientific projections in order to mitigate the specific negative health impacts of climate change to the area. Some populations will face greater challenges than others due to variations in severity of weather events and differences in financial, and healthcare resources. Knowing that infectious diseases do not restrict themselves within an area or population group, developed countries should work together with the more vulnerable, less-developed countries, with the aim of reducing the exposure to climate change related health threats.[7]

Healthcare workers, in particular, serve a vital role in mitigating this problem. Public health officials must establish surveillance for unusual or drug resistant diseases, help inform clinicians about appropriate antimicrobial use, and advise on national programmes for disease control. Hospitals must ensure sufficient laboratory capacity and funding to investigate new agents and develop plans for handling infection outbreaks. National and international political commitment is also necessary for rapid containment of this global issue.[24]

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Cover Picture:
'Impulse'
Acrylic on canvas
By Pierre Mallia

Pierre Mallia started painting when he was young watching with his father; in the last few years he took up painting again and focuses on Maltese views, mountains, ships and animals. This cover painting was a spontaneous one which gave him the 'impulse' to start painting again.

Original Article

Maternal risks associated with pregnancy in women with advanced maternal age

Mandy Collict, Yves Muscat Baron, Miriam Gatt, Neville Calleja

Abstract

Introduction: The trend towards delayed motherhood has accelerated in developed countries over the last few decades. Advanced maternal age (AMA) is defined as age 35 years and older at the estimated date of delivery.

Objective: The aim of this large retrospective cohort study is to assess for the association between AMA and adverse maternal outcomes after adjustment for confounding factors in maternal characteristics and in the obstetric history.

Study Design: Mothers of 20 years and older, who delivered singleton babies in Malta and Gozo between 1st January 2000 and 31st December 2014 were studied. All data was derived from the National Obstetric Information System.

Results: The study population included 55,943 singleton births. 12.2% (6,838) of mothers were between 35 – 39 years and 2.4% (1,325) were 40 years and older. Significant difference was found between maternal age and BMI (p<0.0001), maternal smoking status (p<0.0001), non-insulin dependent diabetes mellitus (p=0.004), history of stillbirth (p<0.0001), gestational diabetes (p<0.0001), pregnancy – induced – hypertension (p=0.008) and pre-eclampsia (p=0.008). Significant difference was also found between maternal age and mode of delivery (p<0.0001). Regression analysis revealed persistent significant differences between maternal age and different maternal outcomes.

Conclusion: This study demonstrates that AMA in Malta significantly increases the risk for hypertension in pregnancy, gestational diabetes and caesarean delivery. Care providers need to be aware of these increased risks and adjust their obstetric management according to the individual to ensure optimal maternal outcomes.

Mandy Collict* MD, MSc (Reproductive Health), MRCSEd
Department of Obstetrics & Gynaecology
Mater Dei Hospital
Msida, Malta
mandy.caruana@gov.mt

Yves Muscat Baron MD, FRCOG, FRCPI, PhD
Department of Obstetrics & Gynaecology
Mater Dei Hospital
Msida, Malta

Miriam Gatt MD, MSc (Public Health Medicine), MSc.HSCR(Sheffield)
Department of Health Information and Research

Neville Calleja MD, PhD, FFPH, CStat
Department of Health Information and Research

**Corresponding Author*

Keywords

Malta, Pregnancy induced Hypertension, Gestational Diabetes, Maternal Age

Introduction

The trend towards delayed childbearing has accelerated in developed countries over the last three decades.[1] Advanced maternal age (AMA) is commonly defined as age 35 years an older at the estimated date of delivery. The reasons motherhood is postponed are manifold. Women's pursuit of higher education, effective contraception, advances in assisted reproductive technology, delayed marriage, and longer life expectancy have all been mentioned as possible reasons for this phenomenon.[1-4] Women should be supported in their decisions of whether to have children or not and when to plan childbearing. Accordingly, women with AMA need to be counselled regarding how fertility and pregnancy outcomes change with age.

Published studies and data on the obstetric risks associated with childbirth at 35 years or over are various and inconsistent. AMA continues to be associated in various numbers of studies with a range of adverse pregnancy outcomes including antepartum haemorrhage, malpresentation, low birth weight, pre-term birth, stillbirth, operative vaginal delivery and increased rates of Caesarean deliveries.[5-6] However, other studies have yielded inconsistent conclusions about both the specific outcomes adversely affected by maternal age and the strength of their association.[7-8]

The only published study on AMA and pregnancy outcomes in Malta was back in 1987 by Savona Ventura and Grech on risks factors in elderly obstetric patients, in the European Study Group on Social Aspects of Human Reproduction, V annual meeting.[3] The scope of this research is to present a clearer picture of what the actual risks to these Maltese elderly women are and also help to compare our findings with that of other countries.

Methodology

This research project is a Retrospective Cohort Study which analyses maternal complications associated with AMA.

All data was derived from the National Obstetric Information System (NOIS) of Malta. This research analyses almost all deliveries in Malta and Gozo over a fifteen-year period from the year 2000 to 2014. The study population was recruited from the NOIS according to the following inclusion and exclusion criteria.

Inclusion criteria:

- Mothers of 20 years of age and over
- Who delivered singletons babies, live births or even still births
- In Malta or in Gozo
- Between 1st January 2000 and the 31st of December 2014

Exclusion criteria:

- Mothers of less than 20 years
- Mothers with multiple pregnancies
- Missing maternal age

All the data analysis was performed by the Statistical Package for the Social Sciences.

Results:

Between the year 2000 and the year 2014, a total of 61,365 deliveries were registered in Malta and Gozo (Figure 1).

1. Demographic Data

All women studied were divided into 5 maternal age groups (Table 1). The average maternal age was 29.2 years (range 20 years – 55 years) and the median age was 29 years. Figure 2 represents the maternal age over this 15-year period.

The maternal weight at booking visit and height were also analysed and the body mass index (BMI) was worked out. In the studied population, 3.2% (1,258 mothers) were underweight, 53.8% (21,019 mothers) had normal BMI, 26.4% (10,324 mothers) were overweight and 16.5% (6,450 mothers) were obese.

The data was analysed by the Medical Statistician (NC) in the employ of the Department of Health Information and Research. The Pearson Chi- Squared (PCS) test was used to assess the significant difference between maternal age and BMI and the results were statistical significant $p<0.0001$. It was noted that although the most common BMI was the normal BMI, a large percentage of mothers were overweight and obese (Figure 3).

***Figure 1**: Study plan*

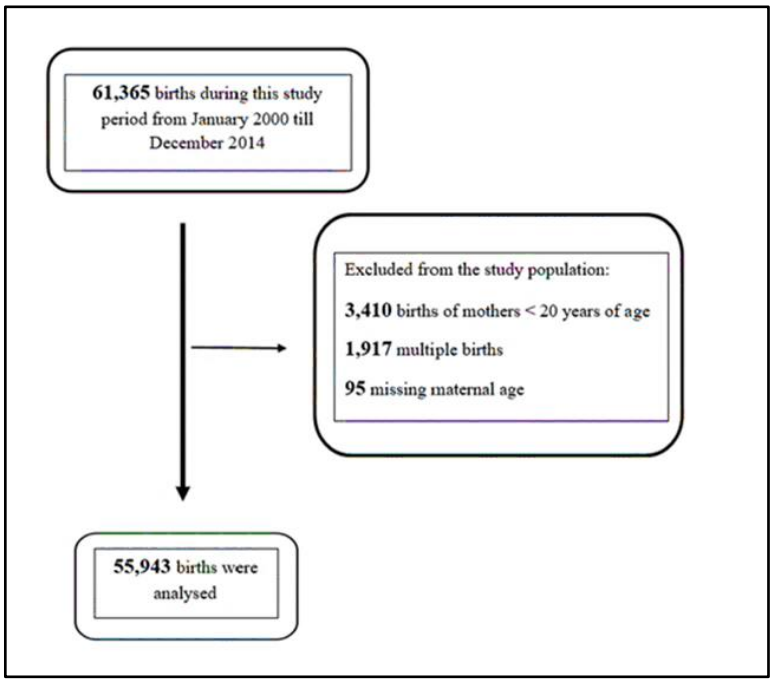


***Table 1**: Patient Age Distribution*

Maternal Age Groups	Number of Mothers	Percentage of Mothers
20 – 24 years	9,814	17.5%
25 – 29 years	20,417	36.5%
30 – 34 years	17,549	31.4%
35 – 39 years	6,838	12.2%
≥ 40 years	1,325	2.4%

***Figure 2**: Trends in maternal age in Malta from 2000 - 2014*

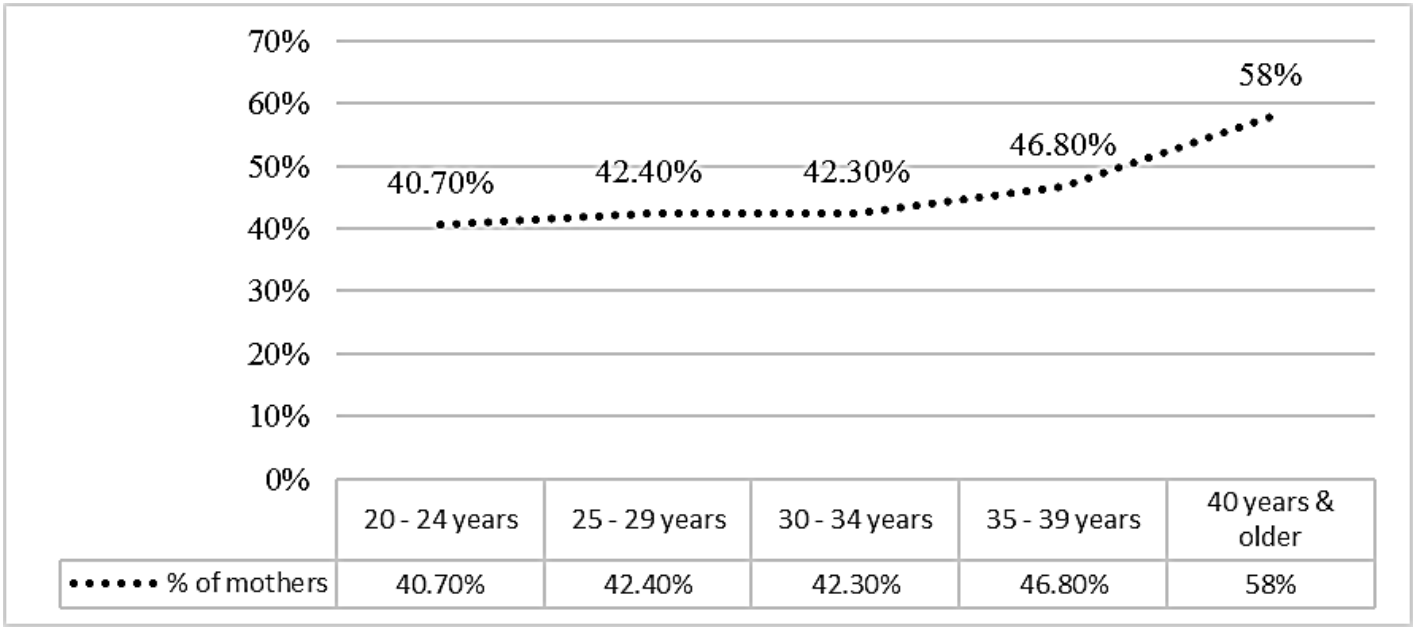
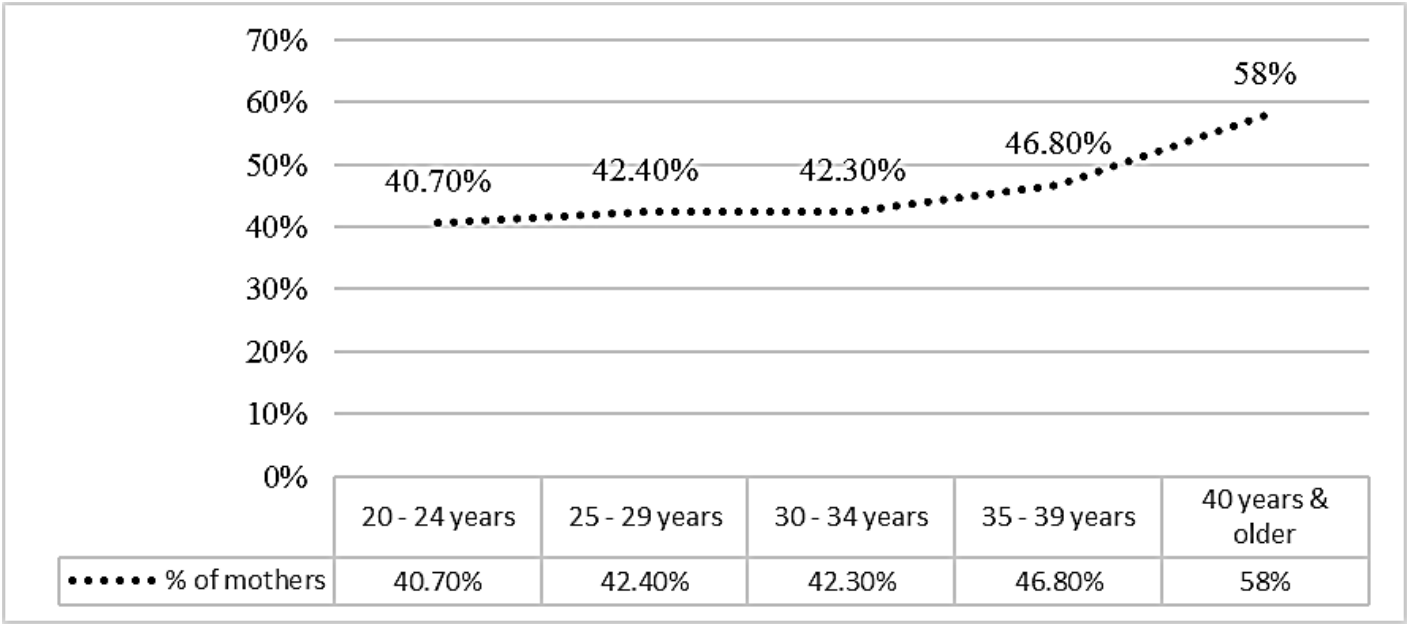


***Figure 3**: The percentage of mothers with BMI > 25 and maternal age*



2. Maternal Complications

As part of this study, the pathology that arises in pregnancy was also studied and specifically we looked into gestational diabetes and hypertension in pregnancy.

For gestational diabetes, mothers were further divided into those who were treated without insulin and those who were treated with insulin. The results show that as the maternal age increase, the percentage of mothers that developed gestational diabetes increases (Table 2). The PCS test was used to analyse the effect of age on the results obtained and the results were statistically significant (p<0.0001).

The data was further analysed by regression analysis (Table 3). A forward stepwise logistic regression model was used and the significant confounders were maternal age (p<0.0001), parity (p<0.001) and BMI (p<0.0001).

As regards hypertension in pregnancy, pregnancy induced hypertension, pre-eclampsia and eclampsia were specifically studied in the 5 different age groups (Table 2). In pregnancy induced hypertension, a sharp rise is noted in the advanced maternal age groups. PCS test was used and the results were statistically significant (p<0.0001). Pre-eclampsia was also much commoner in the elderly age groups. In the elderly age groups, 1.0% of mothers had pre-eclampsia (p=0.008). There were only 12 cases of eclampsia reported in this 15-year study period (p=0.668).

Further data analysis was carried out on hypertension in pregnancy (Table 4). Forward stepwise logistic regression analysis was also carried out. In this latter analysis pregnancy – induced hypertension, pre-eclampsia and eclampsia were grouped into this single group – hypertension in pregnancy. The significant confounders for this model were maternal age (p<0.0001), parity (p<0.0001), IDDM (p<0.0001), cigarette smoking (p=0.01), and BMI (p<0.0001).

***Table 2**: Gestational diabetes, Pregnancy induced hypertension, pre-eclampsia, eclampsia and maternal age*

Maternal Age Group	**20 – 24 years**	**25 -29 years**	**30 – 34 years**	**35 – 39 years**	**≥ 40 years**
Diagnosis of Gestational Diabetes					
No	98.3% (9,646)	97.3% (19,872)	96.7% (16,974)	95.9% (6,555)	94.4% (1,251)
Yes: not treated with insulin	1.5% (144)	2.3% (475)	3.0% (531)	3.9% (264)	5.3% (70)
Yes: treated with insulin	0.0% (0)	0.1% (12)	0.1% (9)	0.0% (1)	0.0% (1)
Diagnosis of Pregnancy Induced Hypertension					
No	94.4% (9,260)	94.1% (19,206)	94.1% (16,519)	92.9% (6,350)	89.0% (1,179)
Yes	5.4% (526)	5.6% (1,147)	5.7% (993)	6.9% (471)	10.9% (144)
Diagnosis of Pre-Eclampsia					
No	99.1% (9,724)	99.2% (20,246)	99.2% (17,401)	98.8% (6,755)	98.9% (1,310)
Yes	0.7% (64)	0.5% (111)	0.7% (116)	1.0% (69)	1.0% (13)
Diagnosis of Eclampsia					
No	99.7% (9,785)	99.7% (20,349)	99.8% (17,511)	99.8% (6,822)	99.7% (1,321)
Yes	0.0% (2)	0.0% (4)	0.0% (3)	0.0% (2)	0.1% (1)

Table 3: *Adjusted results for gestational diabetes and maternal age*

Gestational Diabetes	Adjusted Rates	Standard Error	Confidence Interval (Lower)	Confidence Interval (Upper)
20 – 24 years	3%	0.005	0.02	0.04
25 – 29 years	4%	0.007	0.03	0.06
30 – 34 years	6%	0.009	0.04	0.08
35 – 39 years	7%	0.011	0.05	0.09
40 years & older	9%	0.017	0.06	0.13

Table 4: *Results for Hypertension in Pregnancy*

Hypertension in Pregnancy	Adjusted Rates	Standard Error	Confidence Interval (Lower)	Confidence Interval (Upper)
20 – 24 years	7%	0.013	0.05	0.10
25 – 29 years	7%	0.013	0.05	0.10
30 – 34 years	7%	0.013	0.05	0.10
35 – 39 years	9%	0.015	0.06	0.12
40 years & older	11%	0.021	0.08	0.16

Figure 4: *Maternal age groups and their mode of delivery*

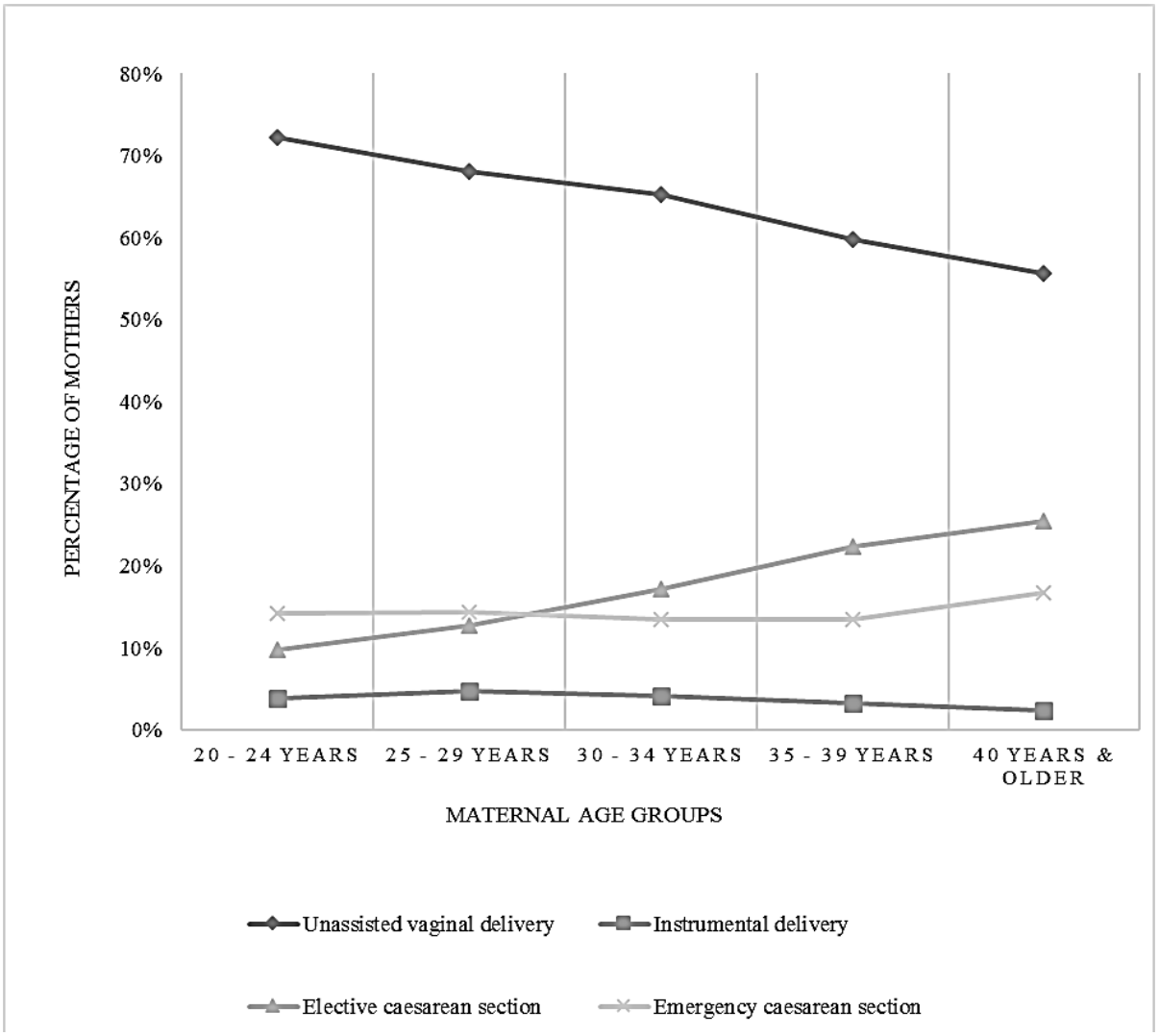


Table 5: *Adjusted results for Mode of Delivery and Maternal Age*

Mode of Delivery	Normal Vaginal Delivery OR (95% CI)	P-value	Instrumental Delivery OR (95% CI)	P-value	Elective Caesarean Section OR (95% CI)	P-value
20 – 24 years	2.507 (2.117 – 2.968)	< 0.0001	1.346 (0.899 – 2.015)	0.149	0.868 (0.713 – 1.056)	0.158
25 – 29 years	2.070 (1.760 – 2.436)	< 0.0001	1.761 (1.189 – 2.607)	0.005	0.965 (0.802 – 1.161)	0.706
30 – 34 years	1.716 (1.458 – 2.020)	< 0.0001	1.860 (1.256 – 2.755)	0.002	1.056 (0.879 – 1.269)	0.558
35 – 39 years	1.247 (1.050 – 1.480)	0.012	1.562 (1.037 – 2.353)	0.033	1.036 (0.854 – 1.256)	0.722
40 years & older	Reference		Reference		Reference	

3. Mode of Delivery

In this study, the mode of delivery was also analysed (Figure 4). The PCS test was used to analyse the effect of maternal age on the mode of delivery and the results were statistically significant ($p<0.0001$).

For the mode of delivery, forward stepwise multinomial regression analysis was carried out (Table 5). The significant confounders were maternal age ($p<0.0001$), year of delivery ($p<0.0001$), IDDM ($p<0.0001$), parity ($p<0.0001$), cigarette smoking ($p<0.0001$), BMI ($p<0.0001$) and history of stillbirth ($p<0.0001$).

Discussion

In the Western world, the average maternal age at which mothers are giving birth is continually rising. Risks associated with pregnancy in women of AMA have been addressed in numerous studies, mostly focusing on the medical risks associated with advancing maternal age. The main purpose of this study was to analyse the Maltese local data. The importance of this research lies in the fact that it is the first recent research of its kind carried out locally after the study published by Savona-Ventura and Grech in 1987.[3]

In the present study, a significant increase in medical complications were noted in mothers with AMA. A significant increase was noted in pregnancy – induced hypertension ($p<0.0001$), pre – eclampsia ($p=0.008$) and gestational diabetes ($p<0.0001$). Similar findings were noted in other studies carried out in Europe,[9-10] in Asia,[11-12] and in USA.[13-14]

The regression analysis carried out showed that hypertension in pregnancy increased steadily with age. A sharp rise in hypertension was seen after the 35 years. For gestational diabetes, a steady increase in mothers with advancing age was noted after the effect of the significant confounders was removed.

With advancing age, pancreatic B cell function and insulin sensitivity diminish, increasing risk in developing type 2 diabetes.[15] Due to the diminished pancreatic B cell response to glycaemic stimulation women with AMA may be more insulin-resistant than younger women, which, when combined, make gestational diabetes more likely. The presence of gestational diabetes increases the risk for foetal macrosomia.[16] Inherently the Maltese population as in most Mediterranean populations is at risk of gestational diabetes which is further augmented in women with AMA.[17]

There is increased risk of gestational hypertensive disorders with AMA.[18] It has been hypothesized that haemodynamic response to pregnancy and ageing might be associated with differences in blood pressure levels during pregnancy between younger and older women.[19] Maternal blood pressure is associated with impaired foetal growth during pregnancy especially during the third trimester of pregnancy and increased risks of adverse outcomes.[20]

Pregnant women experiencing hypertensive disorders are also at greater risk of stillbirth.[21] This risk of foetal demise is further exacerbated in hypertensive women in the AMA cohort.[22] The risk of stillbirth is increased with the onset of pre-eclampsia, and pre-eclampsia is also more prevalent in women with AMA.[23]

Overarching the risks of women in the AMA group for both gestational diabetes and hypertension is the increased prevalence of high BMI. In this study 26.4% of pregnant women were overweight and 16.5% were obese. The combination of AMA and an elevated BMI increase the risk for the occurrence of gestational diabetes.[24] Similarly, hypertensive disorders and pre-eclampsia are associated with AMA and an elevated BMI.[25] Moreover the combination of AMA, elevated BMI, diabetes and hypertension have a multiplier effect on increasing the risk of adverse maternal and neonatal outcomes.[26-27]

In this research, statistical significant results were found between mode of delivery and the maternal age ($p<0.0001$). The percentage of women delivering by normal vaginal delivery was noted to decrease with advancing age, while the percentage of women delivering by elective caesarean sections was noted to increase with advancing age. In mothers over 35 years, an increase was also noted in the rate of emergency caesarean sections. The regression analysis carried out confirmed that these results were still noted after the effects of the other significant confounders were removed. Several studies published similar findings of high rate of caesarean sections in different countries from the year 1987 to the year 2014.[7, 28-31]

In contrast with other international studies, in this study population, instrumental deliveries were noted to decrease with AMA. Wang et al reported in their study carried out in Norway, that

instrumental deliveries were increased in nulliparous women.[7] Tan and Tan also reported in 1994, that the incidence of instrumental deliveries was higher in women over the age of 35 years.[29]

In this study the trend of increased elective caesarean section avoiding vaginal delivery and more so instrumental assistance suggests that obstetricians in this unit may have a lower threshold for intervention in pregnant women in AMA cohort. This is especially noted in nulliparous women with AMA. The obstetrician's decision to resort to elective caesarean section in women with AMA would be further catalysed in the presence of medical complications. Breech presentation also increases with AMA contributing to an increased caesarean section in this cohort of women.[32] In an effort to reduce the risk of sudden stillbirth in the postdates period, resort to induction of labour and subsequent failure to progress in women with AMA may also increase the rate of abdominal delivery.[33, 34] Maternal requests in cases of AMA possibly compounded by a period of subfertility may be another reason for of increased elective caesarean section.[35]

Conclusion

This study demonstrates that advanced maternal age in Malta significantly increases the risk for hypertension in pregnancy, gestational diabetes and caesarean delivery.

With the persistent rise in the number of mothers having their children at advanced age in Malta, the set- up of a multidisciplinary specialized clinic for pre-conception, antenatal and postnatal care for these mothers may prove useful in improving outcome in pregnancies of women with AMA.

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Original Article

CYP2C19 genetic polymorphisms in Maltese patients on clopidogrel therapy

Francesca Wirth, Graziella Zahra, Robert G Xuereb, Christopher Barbara, Liberato Camilleri, Albert Fenech, Lilian M Azzopardi

Abstract

Introduction and Aims: The CYP2C19 enzyme is involved in the metabolism of various therapeutically-important drugs including clopidogrel.

The aims were to determine *CYP2C19 *2* and **17* variant allele frequencies and *CYP2C19* genotype distribution in a cohort of Maltese patients on clopidogrel and to compare observed frequencies of the *CYP2C19 *2* allele and **2/*2* genotype in this cohort to other populations bordering the Mediterranean Sea.

Methods: *CYP2C19 g*enotyping in a cohort of Maltese patients on clopidogrel was performed using TaqMan™ Drug Metabolism Genotyping Assays. Frequencies of the *CYP2C19 *2* and **17* variant alleles and six genotypes (**1/*1*, **1/*2*, **2/*2*, **1/*17*, **17/*17*, **2/*17*) were determined. Observed frequencies of the **2* allele and **2/*2* genotype were compared to fourteen populations bordering the Mediterranean Sea.

Results: Frequency of the *CYP2C19 *2* and **17* allele in the 244 Maltese patients genotyped was 12.3% and 15.4% respectively. *CYP2C19* genotype distribution was: **1/*1* (52.1%), **1/*17* (22.5%), **1/*2* (18.0%), *2/*17* (6.6%), **17/*17* (0.8%) and *2/*2 (0). There was no statistically significant difference in **2* allele frequency between the Maltese cohort and all fourteen populations bordering the Mediterranean Sea.

Conclusions: This study reports the frequency of *CYP2C19 *2* and **17* variant alleles in a cohort of Maltese patients treated with clopidogrel. The high percentage of patients genotyped as carriers of the **2* (25%) or **17* (23%) variant alleles indicates that CYP2C19 genotyping could be used to guide clinicians in the individualisation of antiplatelet therapy.

Francesca Wirth* BPharm (Hons), MPhil, PhD
Department of Pharmacy,
Faculty of Medicine and Surgery,
University of Malta,
Msida, Malta
francesca.wirth@um.edu.mt

Graziella Zahra DMedSc
Department of Pathology,
Mater Dei Hospital,
Msida, Malta

Robert G. Xuereb MD FRCP(L) FRCP(E) FESC FACC
Department of Cardiology,
Mater Dei Hospital,
Msida, Malta

Christopher Barbara MD, MSc (Lond), DLSHTM, FMCPath
Department of Pathology,
Mater Dei Hospital,
Msida, Malta

Liberato Camilleri BEd(Hons), MSc, PhD(Lanc)
Department of Statistics and Operations Research,
Faculty of Science,
University of Malta,
Msida, Malta

Albert Fenech MD, FRCP, FESC, MOM
Department of Cardiology,
Mater Dei Hospital,
Msida, Malta

Lilian M. Azzopardi BPharm (Hons), MPhil, PhD, MRPharmS, FFIP
Department of Pharmacy,
Faculty of Medicine and Surgery,
University of Malta,
Msida, Malta

**Corresponding Author*

Keywords

clopidogrel; *CYP2C19* polymorphisms; drug metabolism; Maltese; Mediterranean

Introduction

The CYP2C19 enzyme is involved in the metabolism of a number of therapeutically-important drugs, including the thienopyridine inactive prodrug

clopidogrel. Biotransformation in the liver is required to form the pharmacologically active metabolite of clopidogrel, which selectively and irreversibly antagonises the $P2Y_{12}$ component of the adenosine diphosphate receptor on the platelet surface, consequently attenuating platelet aggregation.[1] Two sequential hepatic oxidative steps are involved in clopidogrel bioactivation[1] and CYP2C19 is the principal enzyme involved in both steps.[2] *CYP2C19* single nucleotide polymorphisms have been identified as significantly and consistently being associated with variability in clopidogrel response.[3-5] Identifying patients' genotype and ability to effectively transform clopidogrel to the active metabolite is crucial for individualisation of treatment in cardiology.

The cytochrome P (CYP) 450 isoenzyme 2C19 (CYP2C19) is highly polymorphic and more than 30 variant alleles have been identified.[6] The *CYP2C19* '**1*' 'wild-type' allele is associated with normal 'functional' CYP2C19-mediated metabolism and is assigned when variant alleles are not identified. The '**2*' variant allele is the most prevalent loss-of-function allele which translates into decreased drug metabolism and the '**17'* allele is a gain-of-function allele which may result in increased activity due to enhanced expression.[3]

Frequencies of the *CYP2C19 *2* and **17* alleles in forty-one healthy Maltese volunteers have been reported.[7] The aims of this study were to determine the frequency of the *CYP2C19 *2* and **17* alleles and *CYP2C19* genotype distribution in a cohort of Maltese patients on clopidogrel and to compare the frequencies of the **2* allele and **2/*2* genotype observed in this cohort to other populations bordering the Mediterranean Sea.

Methodology

Ethics approval

The study protocol was approved by the University of Malta Research Ethics Committee.

Study design and setting

This cohort study was undertaken at Mater Dei Hospital. Patients were prospectively identified from the cardiac catheterisation suite at the Department of Cardiology and *CYP2C19* genotyping was performed at the Molecular Diagnostics Unit of the Department of Pathology.

Patient recruitment and sample collection

Maltese patients ≥ 18 years undergoing percutaneous coronary intervention (PCI) with stent placement and prescribed dual antiplatelet therapy with aspirin and clopidogrel were recruited by non-probability sampling over a twelve-month period (January-December 2014). The advantages of this sampling method are that it is cost and time-effective. Although non-probability sampling does not guarantee that each patient has equal probability of being selected, the sample is a good representation of the population since the patients were recruited over a one-year period. After obtaining written informed consent, 5 mL of peripheral blood was collected from each patient in a purple-top ethylenediaminetetraacetic (EDTA) vacutainer at the time of PCI.

Genomic DNA extraction and CYP2C19 genotyping

Genomic DNA was extracted from 200 µL of the EDTA-blood sample using the QIAamp® DNA Mini Kit on the fully automated QIAcube *(Qiagen)*. *CYP2C19* genotyping for the **2* (rs4244285) and **17* (rs12248560) alleles was performed with TaqMan™ Drug Metabolism Genotyping Assays *(Thermo Fisher Scientific)*, which involve DNA amplification and homogeneous solution hybridisation using fluorescence resonance energy transfer, on the 7500 ABI real-time polymerase chain reaction (PCR) system *(Applied Biosystems)*. Each well in the PCR plate had a final reaction volume of 25 µl consisting of gDNA, an allele-specific probe labelled with VIC® dye and another with 6FAM™ dye, forward and reverse primers and TaqMan™ Universal PCR Master Mix *(Thermo Fisher Scientific)*. Thermal cycling conditions consisted of initial denaturation at 95 °C for 10 minutes, followed by 50 denaturation cycles at 92 °C for 15 seconds and annealing/extension at 60 °C for 90 seconds. Patients were genotyped as homozygous (**1/*1, *2/*2, *17/*17*) or heterozygous (**1/*17, *1/*2, *2/*17*) for the *CYP2C19* alleles.

Categorisation of patients into metaboliser phenotypes for clopidogrel

The observed genotypes were classified into four clopidogrel metaboliser phenotypes according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *CYP2C19*

genotype and clopidogrel therapy[4], namely extensive metabolisers - EMs (**1/*1*), ultra-rapid metabolisers - UMs (**1/*17*, **17/*17*), intermediate metabolisers - IMs (**1/*2*, **2/*17*) or poor metabolisers - PMs (**2/*2*). The caring cardiologists were informed of the genotype and phenotype results.

Comparison of CYP2C19 polymorphisms in populations bordering the Mediterranean Sea

The observed frequencies of the *CYP2C19 *2* allele and **2/*2* genotype in the Maltese patient cohort on clopidogrel were compared to fourteen populations bordering the Mediterranean Sea, namely Albanian, Bosnian, Croatian, Egyptian, Greek, Israeli, Lebanese, Moroccan, Slovenian, Southern French, Southern Italian, Southern Spanish, Tunisian and Turkish populations.

Statistical analysis

IBM SPSS Statistics 24 was used for statistical analysis. Observed and expected *CYP2C19* genotype frequencies were compared using the Hardy-Weinberg (H-W) equilibrium calculation. The Fisher's exact test was used to determine whether the observed data supports the null hypothesis that the cohort is in H-W equilibrium by adopting a 0.05 level of significance. Observed proportions of the *CYP2C19 *2* allele and **2/*2* genotype in the study cohort were compared to the fourteen populations bordering the Mediterranean Sea using the difference of two proportions z-test. A p-value less than 0.05 indicates that the proportions differ significantly, while a p-value greater than 0.05 indicates a non-significant (NS) difference, hence comparable proportions.

Results

Two hundred and forty-four (29%) Maltese patients on clopidogrel (75% male, mean age 65.43 ±1.24 years, all Caucasian, 45% undergoing PCI following admission with acute coronary syndrome) out of the total 843 Maltese and non-Maltese patients who underwent PCI from January to December 2014 were genotyped for the *CYP2C19 *2* and **17* alleles.

*CYP2C19 *1*, **2* and **17* allele frequencies were 72.3%, 12.3% and 15.4% respectively. *CYP2C19* genotype distribution of the 244 patients was **1/*1* (52.1%), **1/*17 (*22.5%), **1/*2* (18.0%), **2/*17* (6.6%) and **17/*17* (0.8%). No patients were genotyped as **2/*2*. Since there was a discrepancy between the observed frequencies and the corresponding expected frequencies, particularly for the **2/*17*, **2/*2* and **17/*17* genotypes, the Fisher's exact p-value obtained (0.051) is very close to the 0.05 threshold for H-W equilibrium (Table 1).

Table 1*: Observed and expected CYP2C19 genotypes (N=244)*

CYP2C19 **genotype**	**Observed number (%)**	**Expected number (%)** *H-W*	**X^2; p-value** *(Fisher's exact test)* *H-W*
**1/*1*	127 (52.1)	127.5 (52.3)	$X^2(5) = 11.04$; p=0.051
**1/*17*	55 (22.5)	54.3 (22.3)	
**17/*17*	2 (0.8)	5.8 (2.4)	
**1/*2*	44 (18.0)	43.4 (17.8)	
**2/*17*	16 (6.6)	9.3 (3.8)	
**2/*2*	0 (0)	3.7 (1.5)	

H-W: Hardy-Weinberg

***Table 2**: Distribution of CYP2C19 *2 allele and *2/*2 genotype: Maltese cohort compared to other populations bordering the Mediterranean Sea*

Population	Number of patients (number of alleles)	Frequency % (*p*-value)	Frequency % (*p*-value)
		*CYP2C19 *2*	*CYP2C19 *2/*2*
Maltese *Present study*	**244 (488)**	**12.3**	**0**
Albanian[8]	40 (80)	20.0 (NS)	2.5 **(S)**
Bosnian[9]	77 (154)	16.9 (NS)	2.6 **(S)**
Croatian[10]	200 (400)	15.0 (NS)	3.0 **(S)**
Egyptian[11]	247 (494)	10.9 (NS)	0.8 (NS)
Greek[12]	283 (566)	13.1 (NS)	2.1 **(S)**
Israeli[13]	140 (280)	15.0 (NS)	2.9 **(S)**
Lebanese[14]	161 (322)	13.4 (NS)	3.1 **(S)**
Moroccan[15]	290 (580)	11.4 (NS)	0.3 (NS)
Slovenian[16]	129 (258)	15.9 (NS)	0.8 (NS)
Southern French[17] *(Marseille, Nimes)*	213 (426)	12.0 (NS)	1.0 (NS)
Southern Italian[18] *(Messina)*	360 (720)	11.1 (NS)	1.7 **(S)**
Southern Spanish[19] *(Valencia)*	362 (724)	13.1 (NS)	1.9 **(S)**
Tunisian[20]	100 (200)	11.5 (NS)	0 (NS)
Turkish[21]	404 (808)	12.0 (NS)	1.0 (NS)

S – significant; NS - not significant

When classifying the patients according to metaboliser phenotype relative to clopidogrel, 52.1% of the patients were EMs, 24.6% were IMs, 23.4% were UMs and no patients were PMs.

Frequencies of the **2* allele ranged from 10.9% in Egyptians to 20% in Albanians (Maltese patients 12.3%). Prevalence of the **2* allele in the Maltese cohort is comparable (NS) to all fourteen populations bordering the Mediterranean Sea. Frequencies of the **2/*2* genotype ranged from 0% in Tunisians to 3.1% in Lebanese (Maltese patients 0%). Prevalence of the **2/*2* genotype in the Maltese patient cohort is comparable (NS) to six populations bordering the Mediterranean Sea, namely Egyptian, Moroccan, Southern French, Slovenian, Turkish and Tunisian populations (Table 2).

Discussion

This is the first report on the frequency of *CYP2C19 *2* and **17* genetic polymorphisms in Maltese patients on clopidogrel therapy.

The frequency of the *CYP2C19 *2* allele in this cohort of Maltese patients taking clopidogrel (12.3%) is lower than the reported prevalence in healthy Maltese volunteers (20%)[7] and in Europeans and Africans (18%).[22] The **2* allele frequency in the patient cohort studied is comparable to the fourteen populations bordering the Mediterranean Sea included in the comparison since no statistically significant difference was observed.

The reported prevalence of the *CYP2C19 *17* allele in healthy Maltese volunteers (26%)[7] and in Europeans and Africans (22.4% and 23.5% respectively)[22] is higher than the frequency observed in this Maltese patient cohort (15.4%). The prevalence of the *CYP2C19 *17* allele was studied in three populations bordering the Mediterranean Sea, namely Southern French[17], Southern Spanish[19] and Greek[23], with a higher observed frequency (20%) compared to the Maltese cohort (15.4%). However, the difference was not statistically significant for all three populations.

Prevalence of *CYP2C19* PMs is reported to be between 1 and 7% in Caucasians and Africans.[4,24,25] In Europe, a north-south gradient, with a decreased prevalence of PMs in Southern Europe, has been observed.[21] No patients in this cohort were genotyped as homozygous for the *CYP2C19 *2* allele and the frequency of the **2/*2* genotype was comparable to only six of the fourteen populations bordering the Mediterranean Sea included in the comparison.

Twenty-five percent of this Maltese patient cohort was genotyped as heterozygous for the *CYP2C19 *2* allele and phenotyped as IMs, while 23% of the patients were phenotyped as UMs. These findings have relevant clinical implications vis-à-vis clopidogrel since these patients are at an increased risk of unwanted outcomes due to compromised clopidogrel activity.

The *CYP2C19 *2* allele is clinically important with respect to clopidogrel and has been associated with reduced formation of active metabolites and higher on-clopidogrel platelet reactivity (PR), leading to increased risk of adverse cardiovascular events in IMs and PMs compared to EMs.[26-29] The strongest association is reported in patients with acute coronary syndrome undergoing PCI with stent placement, where carriers of the **2* allele are at higher risk of stent thrombosis compared to non-carriers.[27,30,31] According to the CPIC guidelines, an alternative $P2Y_{12}$-receptor inhibitor, such as ticagrelor or prasugrel, should be considered in carriers of the **2* allele (25% in this patient cohort) provided there is no contra-indication.[5]

There are mixed results on the clinical relevance of the *CYP2C19 *17* allele with respect to clopidogrel (23% in this patient cohort), where some studies reported lower on-clopidogrel PR, enhanced response to clopidogrel and increased risk of bleeding, while other studies reported no effect of this allele on clinical outcomes.[32-35] The CPIC guidelines recommend standard dosage of clopidogrel in UMs.[5]

The UM phenotype is clinically relevant for other drugs where *CYP2C19* genetic polymorphisms are implicated in variability of interpatient response, such as for proton pump inhibitors (PPIs), tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and voriconazole. Further study is recommended to assess the prevalence and clinical implications of *CYP2C19* genetic polymorphisms in patients taking these drugs.

For PPIs, UMs have shown less effective gastric acid suppression and decreased *Helicobacter pylori* eradication rates, hence an increase in dose is recommended.[36] For TCAs (amitriptyline, clomipramine, doxepin, imipramine, trimipramine) and SSRIs (citalopram, escitalopram, sertraline), the UM phenotype is associated with increased metabolism and risk of sub-optimal response, hence the CPIC guidelines suggest an alternative drug not metabolised by CYP2C19.[37,38] With respect to voriconazole, UMs are less likely to attain therapeutic concentrations with standard dosing and selection of an alternative agent not dependent on CYP2C19 metabolism as primary therapy is recommended.[39]

Conclusion

This study reports the frequency of *CYP2C19 *2* and **17* variant alleles in a cohort of Maltese patients on clopidogrel therapy. The high percentage of patients phenotyped as IMs (25%) indicates that CYP2C19 pharmacogenetic testing could be used to guide clinicians in the individualisation of antiplatelet therapy. This study serves as an example of pharmacogenetic testing to achieve precision medicine.

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Original Article

Indoor climate and its impact on atopic conditions in Maltese school children

Caroline Gouder, Claudia Fsadni, Frank Bezzina, Peter Fsadni, Stephen Montefort

Abstract

Several studies on the prevalence of allergic conditions have identified that allergic conditions are on the increase worldwide. The aim of this study was to assess the effect of classroom humidity and temperature levels on schoolchildren in Malta. Our cohort included 191 children. Standardised ISAAC health questionnaires were answered by the children's parents. Lung function tests, acoustic rhinometry, exhaled nitric oxide (NO), exhaled carbon monoxide (CO) and nasal lavage were performed on the participating children. School building characteristics were also studied.

A significant association was noted between a high relative humidity exposure and nasal cross-sectional areas (p=0.003), and doctor diagnosed allergic rhinitis (p=0.002), indicating the presence of allergic rhinitis, as was increased indoor temperature (p=0.003). Increased indoor temperature was also associated with increased exhaled nitric oxide (FeNO) (p<0.001) indicating uncontrolled asthma. In conclusion, increased classroom temperatures and humidity, both linked to decreased classroom ventilation, were associated with increased incidence of allergic conditions in schoolchildren in Malta. These results emphasize the important need for the introduction of climate control and de-humidifying systems in our schools with the aim of decreasing the prevalence and severity of such conditions in this cohort of patients.

Caroline Gouder* MD MRCP
Department of Respiratory Medicine
Mater Dei Hospital
Msida, Malta
carolinegouder@gmail.com

Claudia Fsadni FRCP MSc
Department of Infectious Diseases
Mater Dei Hospital
Msida, Malta

Frank Bezzina MSc PhD
Faculty of Economics
Management and Accountancy
University of Malta
Msida, Malta

Peter Fsadni FRCP PhD
Department of Respiratory Medicine
Mater Dei Hospital
Msida, Malta

Stephen Montefort FRCP PhD
Department of Respiratory Medicine
Mater Dei Hospital
Msida, Malta

**Corresponding Author*

Background

Several studies on the prevalence of allergic conditions have identified that allergic conditions are on the increase worldwide.[1-3] Therefore, there is ongoing worldwide research to identify possible causative agents so as to be able to provide advice to patients regarding allergen avoidance measures, improvement of environmental and lifestyle factors as well as to individualize treatment accordingly.

Indoor air temperature and humidity levels and their association with allergic conditions, particularly asthma in school-age children, have been investigated in several studies. However, most studies have been performed in households[4-9] and few in classrooms.[10-11] The short-term (0–14 days) health effects of low outdoor temperatures have been well studied in large populations over long time periods. In particular, the association of low outdoor temperatures with respiratory diseases is well established. In these outdoor studies, outdoor temperatures are assumed to be a measure of personal exposure to temperature, but indoor exposure is clearly more important, because people

spend a more substantial part of their lifetime indoors,[5] especially children in the classroom. Building construction characteristics have also been studied and identified to have a causal relationship with the development of allergic conditions in children.[12] Furthermore, these results cannot be extrapolated to small island nations like Malta due to the high population density and unique geographical and environmental characteristics.

Objectives

The primary outcome measure of this study was to identify whether indoor classroom temperature and humidity have an impact on the incidence of allergic disease, respiratory symptomatology as well as lung function in primary schoolchildren.

Materials and Methods

Data collection

Five primary state schools in Malta were selected randomly from five geographical clusters of schools designated north (Fgura), south (Birzebbuga), central and urban (Qormi and Fgura), east and close to sea(Pembroke) and west rural(Dingli). Three classrooms within each school were selected among the 9 to 11year age group with all students asked to participate. The classrooms were randomly selected by ballot system so as to eliminate potential bias.

Ethical approval and consent

This study was approved by the University of Malta Research Ethics Committee and the Education Department Research Directorate.

Health assessment and school characterisation

Standardised ISAAC health questionnaires focusing on wheezing, rhinitis and eczema were answered by the children's parents. Lung function tests using a 'microQuark COSMED' handheld portable spirometer, acoustic rhinometry (A1 Acoustic Rhinometer by GM Instruments) , exhaled NO (NIOX MINO), exhaled CO ('piCO+™ CO' monitor) were performed on the participating children. Characterisation of each participating school was performed focusing on the school building, classrooms, cleaning / maintenance protocols and the outdoor environment.

Physical Parameters

Methods of air sampling were based on the International Organisation for Standardisation (ISO) 16000 series [2*]*.[13] CO_2, temperature and humidity were measured continuously using TSI 7525 IAQ-Calc low-cost loggers. Sampling within the schools took place over a five-day period (Monday morning until Friday afternoon) between November and January. Ventilation rates of classrooms were calculated using the indoor and outdoor CO_2 levels.

Statistical Analysis

Data was transferred to IBM SPSS Statistics version 21 (Inc., Chicago, IL) for analysis. For statistical significance, a *p*-value cut off point of 0.05 was adopted. In the case of multiple comparisons, the Bonferroni correction was applied to avoid inflation of the Type 1 error. Regression analysis was used to eliminate any possible confounding factors.

Results

Out of a total number of 237 pupils in all 15 selected classrooms, 191 (80.59%) consented to taking part in the study. The majority of pupils were female (51.16%) while 48.84% were male and the mean age was 9.56 years (SD 0.58).

Up to 32.98% of all the pupils had wheezing at one time in their life (wheezing ever) while 17.8% had current wheezing in the previous 12 months. Up to 16.75% of all pupils actually had doctor-diagnosed asthma. Symptoms suggestive of allergic rhinitis ever were present in nearly 34% of the children while 29.84% of the children complained of rhinitic symptoms in the previous 12 months. Nasal symptoms in the previous 3 months were highly prevalent among the study population with 40% complaining of a runny nose / nasal phlegm, while half of the pupils complained of a blocked nose. Up to 40% of the pupils complained that current rhinitis interfered with their daily activities. Itchy rashes occurring for at least six months were described in 13.61% of the pupils with 65% of them complaining of symptoms in the previous 12 months. Up to 22% of all children participating in the study had a diagnosis of eczema sometime during their life and this was evenly distributed among all schools.

The mean relative humidity within Maltese classrooms was 62.71%. The mean indoor temperature in all five schools was 18.31 ± 2.23^0C

ranging between a maximum temperature of 22.06^{0}C and a minimum temperature of 14.77^{0}C. The mean 24-hour indoor CO_2 level was 525.21 ppm (SD 300.51 ppm) which is well below the recommended WHO threshold of 1000 ppm (WHO 2010). The mean indoor CO_2 during school hours was 634.32 ppm (SD 201.34ppm). Indoor CO_2 trends showed peak during school hours followed by lower levels when classrooms were unoccupied whilst outdoor CO2 levels were stable (Figure 1). Ventilation rates for each school are shown in (Figure 2). There were significant negative correlations between indoor CO2 concentrations and ventilation rates of the classrooms (r – 0.76 *p* <0.001).

***Figure 1**: Indoor CO_2 levels over 24 hours*

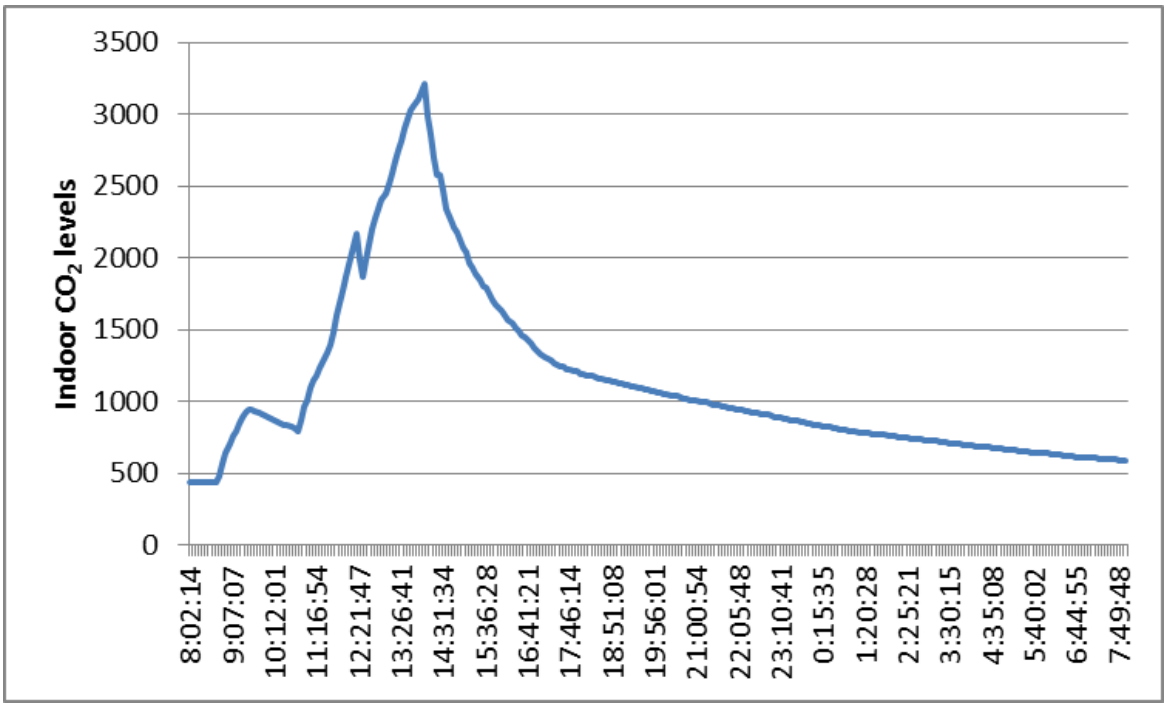
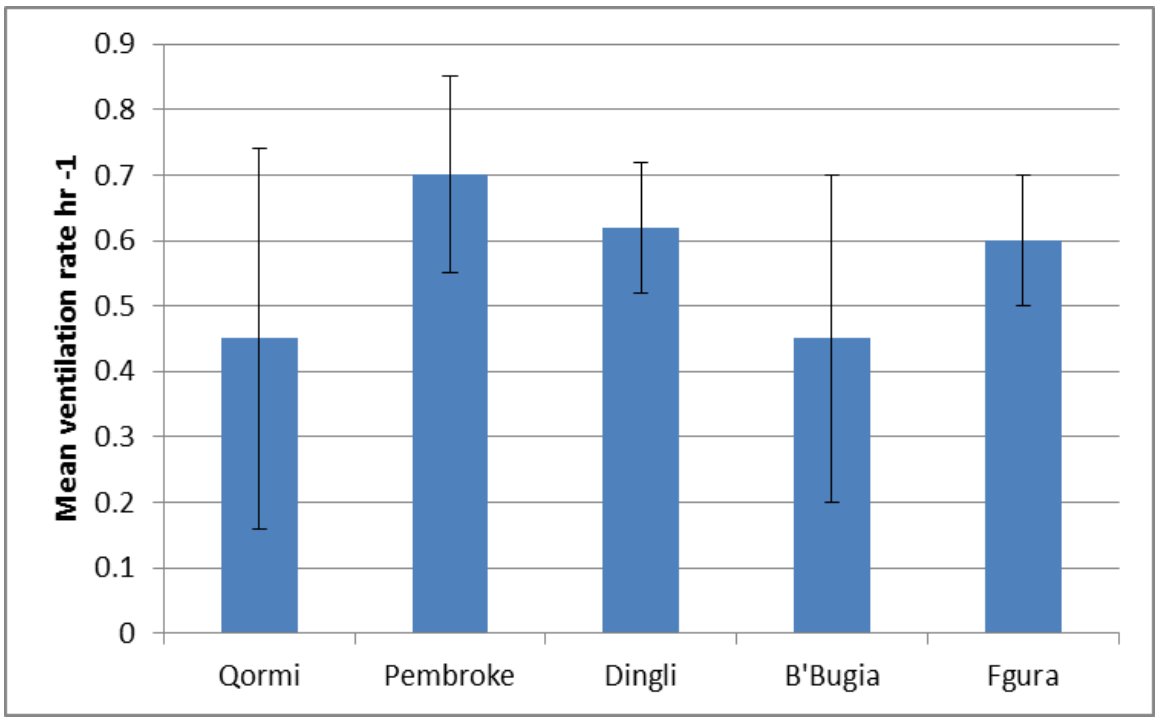


***Figure 2**: Mean indoor ventilation rate per hour*



Association between exposure to environmental parameters and health outcomes

The odds ratios (OR) between symptomatology and environmental exposures was determined by comparing symptoms with high versus low exposure levels to the particular irritant. The high exposure category consisted of pollutant levels equal to or more than the second tertile of concentrations while lower levels of pollutant were defined as low exposure. A logistic regression model was designed so as to eliminate potential confounding factors.

Relative Humidity

Children were less likely to complain of dry throat in the previous three months in classrooms having high humidity levels (OR 0.36, CI 0.14-0.91, p=0.027). A significant association was seen between a high relative humidity exposure and nasal cross-sectional areas (p=0.003) and exhaled CO (p<0.001).

Temperature

Indoor classroom temperatures which were above the third tertile were associated with an increased risk of doctor-diagnosed allergic rhinitis (p=0.002, OR 4.83, CI 1.98-7.56). Increased indoor temperature was associated with a decreased nasal mean cross sectional area in both the right and left nostrils on acoustic rhinometry (p=0.003; OR 4.69; CI 2.36-7.98) as well as an increased FeNO (p<0.001; OR 2.69; CI 1.6-5.65). The mean percentage area of openable class windows was 57%. Class rooms having an openable window area of less than 57% were more likely to have increased indoor temperatures (p=<0.001; OR 1.98 CI 1.27-3.68).

Classroom Ventilation

The mean ventilation in the classrooms was 0.56 per hour (SD±0.19; max. 0.88 and min. 0.12). Indoor ventilation was negatively correlated with indoor temperature (r=-0.55; p=0.0036), therefore the lower the ventilation the higher the indoor temperature. Children in classrooms having ventilation rates within the lower tertile were more likely to complain of current wheezing (p=0.002; OR 3.44 CI 2.4 – 6.5), itchy rashes in the previous 12 months (OR 3.11, CI 1.1-9.13, p=0.043) and doctor diagnosed atopic eczema (p=0.001; OR 3.64 CI 1.65 – 8.6).

Discussion

Studies dating back to the 1970's recommend guidelines on the implementation of indoor climate requirements and improvement of already existing institutions to improve air quality for school children as well as adult staff members alike.[14]

Humidity

High levels of indoor relative humidity were significantly associated with decreased nasal cross-sectional areas and volumes. No studies have linked rhinitis with increasing temperature although a weak association exists with the prevalence of sinusitis.[15] The local findings might be explained by the fact that warm and / or humid classrooms increased the growth of bacteria and fungi thus potentially increasing the exposure levels to these pollutants.

Indoor relative humidity levels should ideally range between 30 and 70%[16] with the mean humidity level within local schools falling within this range (62.71%). Interestingly, all three classrooms in school 3 (Dingli) exceeded the 70% threshold while all five schools had a relative indoor humidity above the SINPHONIE mean of 43%.[17] The indoor/outdoor ratio for relative humidity (0.88) indicated that the outdoor environment was the major determinant of humidity within local schools. For this reason, one potential limitation to these observations could be the fact that monitoring within all the 5 schools was not simultaneous and therefore meteorological conditions could partly explain the humidity levels detected in school 3 (Dingli) especially since sampling took place in December.

Relative humidity readings quantify the extent of saturation of air by water vapour with January characterized by the highest levels of humidity in the Maltese Islands.[18] Analysis of meteorological data has shown that relative humidity during the winter months plays a major role in thermal comfort both indoors and outdoors.[18] Indoor dehumidifiers within individual classrooms should be considered by school administrators so as to control indoor humidity levels thus improving thermal comfort. This is particularly relevant during winter months where decreasing the indoor humidity levels might reduce the extent of class heating (and energy consumption) needed to achieve acceptable thermal comfort levels.

Temperature

Increasing temperature was found to be significantly associated with doctor-diagnosed nasal allergies in our patients. Higher indoor temperature have been suggested to determine sensitisation and development of atopic diseases.[19] In a study by Mi et al in Shangai, performed on 1414 pupils aged between 13 and 14, current asthma and asthma-related symptoms were associated with indoor temperature. The mean temperature in this study was 17^0C (range 13-21^0C), which compared well with our study.[10] In another study of 1000 random children in Edinburgh, ambient temperature (mean 17.8 ± 1.8^0C) and humidity was recorded in 317 bedrooms but no association was identified with respiratory symptoms.[8] Respiratory tract symptoms have been reported to improve both in frequency and severity during summer holidays and weekends especially among children attending moisture-damaged schools.[20] These studies show that ideally, classrooms should have climate control, , so as to avoid development of allergic conditions.

In addition to increased nasal allergies, indoor temperature was associated with a decreased nasal mean cross sectional area in both nostrils, suggestive of allergic rhinitis, when acoustic rhinometry was performed. Changes in nasal cavity volumes were detected by acoustic rhinometry after exposure to an increase in temperature and humidity in a small study of 8 patients by Lal et al.[21] Acoustic rhinometry is a considered to be a safe, non-invasive, objective, and validated measure of nasal obstruction that appears to be of practical use in the diagnosis and management of inflammatory diseases of the upper airways.[22] Introduction of its use for diagnostic purposes might be useful in Malta in certain cases when diagnostic dilemmas are present.

Exhaled NO levels were also raised in association with increased temperatures, suggesting increased airway inflammation. In contrast to our study, Pierse et al identified that a 1^0C increase in temperature (mean bedroom temperature 14.4°C, mean living room temperature 16.53°C.) was associated with a slight overall improvement in lung function, measured using spirometry and peak flow. Exhaled NO was not performed in this study.[5] The effect of temperature on lung function seems to be inconsistent. The prevalence of wheezing in school children was lower in classrooms having higher temperatures,[23] however children were more likely to complain of breathlessness.[10]

Pollutants

Human beings are the main source of indoor CO_2 since they exhale carbon dioxide as a byproduct of metabolism with the average adult's breath having between 35,000 to 50,000 ppm of CO_2.[24] The American Society of Heating, Refrigeration, and Air Conditioning Engineers (ASHRAE) recommends using indoor CO_2 levels to determine the ventilation characteristics of the building. Furthermore, ASHRAE recommends that the mean indoor CO_2 level should not exceed outdoor CO_2 concentrations by more than about 600 ppm.[24] Mean indoor CO_2 levels were below both the recommended WHO threshold of 1000 ppm[25]. A significant negative correlation was found between indoor CO_2 concentration and ventilation rates of the classrooms, confirming usefulness of CO_2 monitoring as a marker of indoor ventilation characteristics.

The characteristics of indoor homes from 280 elementary school students in Romania have also been implicated to cause increased respiratory symptoms in a recent study as part of the SINPHONIE project,[9] related to the limited use of indoor climate control.[9] It has been reported that schools frequently have trouble maintaining indoor relative humidity within the optimum range (30-50 %) recommended for reducing allergens and irritants.[26] Building characteristics have been studied[10,12] and found to contribute to respiratory morbidity with worsened symptoms.[12] Classroom windows of studied schools in Malta could only be half opened. As expected, this resulted in higher indoor temperatures since climate control was not available for use in any of the studied schools. Independently, decreased ventilation was associated with a significant increase in the risk of current wheezing and itchy rashes in the past 12 months as well as an increased risk of doctor-diagnosed atopic eczema. Asthma symptoms are known to be influenced by lack of ventilation in Shangai.[10] Unventilated indoor climate is associated with increased prevalence of asthma, allergic rhinitis and eczema in schoolchildren.[27] Opening windows results in outdoor environmental air pollutants as well as outdoor aeroallergens such as tree and grass pollen entering the classroom. However, closed windows means that indoor allergens such as house

dust mite, animal dander and mould spores are not able to exit the room. When measured, cat and dog allergens were found to be very high in schools,[28] which must be carried on childrens' and staff clothing, emphasizing the need for frequent school cleaning. Sensitisation to indoor allergens has been identified as a major risk factor for the development of childhood asthma several years ago.[19] This is a reasonable finding, particularly in the westernized world since children tend to spend several hours during the day indoors either in their home or in a classroom. House dust mite and mould are determined by their microenvironments. Humidity and warm temperatures tend to favour their growth.[29,30] Single allergens, increased humidity and visible moulds were significantly associated with house dust mite and mugwort pollen.[31] Indoor moulds have been studied in some international studies, to identify whether mould exposure may be linked to childhood respiratory disease. However Celtic identified that mould was not a predisposing factor.[32] In contrast, Yazicioglu et al did identify that fungal counts were higher in the homes of asthmatic children whilst Mi et al observed that indoor moulds were associated with increased asthma exacerbations.[10] Window opening has been described as the only way to remove indoor pollutants.[10] Cooler temperatures could also allow bacteria and viruses to thrive less and result in a reduction in respiratory tract infections.

From our study we can say that it is more advantageous for outside pollutants to enter whilst allowing indoor allergens to exit, but this obviously depends on the surrounding environment where the schools are located. One must keep in mind that elevated levels of air pollutants have also been reported in several studies to be detrimental to respiratory health.[33-35]

Similar studies have also been extrapolated to study teachers, who have been identified to have a higher asthma prevalence than other non-industrial worker groups. Classroom humidity and teachers' respiratory health was explored in North Carolina. Though statistical significance was not reached, there was a modest increase in the risk of respiratory symptoms.[26]

Limitations

The main limitation of the present study is the relatively small number of children participating. CO_2 data interpretations might result in the overestimation of ventilation rates. Investigations for sensitization to aeroallergens, such as dog and cat dander, house dust mite and moulds, would be ideal to complement our findings, since positive results would explain the increased prevalence of allergic conditions in our cohort. Measurement of outdoor pollutants and correlation with ventilation and respiratory symptoms could also provide an explanation to our findings. Fungal counts were not measured in our study.

Conclusion

Higher classroom temperatures and humidity, linked to decreased ventilation, were associated with increased incidence of allergic rhinitis symptoms together with narrowed nasal passages, worsened asthma control and increased atopic eczema. Schools should be advised that classrooms should be well-ventilated, windows opened regularly, possible introduction of mechanical ventilation and dehumidification with the aim of decreasing allergy-associated symptomatology and conditions in children, who spend a significant amount of their childhood years in such an environment. Humidity levels and temperatures should be kept low. Despite our associations, one must keep in mind that causation of allergic conditions is multifactorial, and must be studied on an individual basis.

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Review Article

TIA-like presentations of cerebral amyloid angiopathy

Bernard Galea, Anna Pullicino, Patrick Pullicino

Abstract

Transient focal neurological episodes (TFNEs) are transient ischemic attack (TIA)-like episodes that may occur in patients with cerebral amyloid angiopathy (CAA). The duration of TFNEs is typically similar to TIAs with most symptoms resolving in minutes. Symptoms, similar to those of TIAs include sensory or visual disturbances, motor weakness and language impairment and there may be limb jerking or associated headache. TFNEs have a more gradual onset and tend to spread slowly to contiguous body parts like a migraine aura. TFNEs may occur repeatedly throughout the day and attacks may continue over several months. TFNEs are typically associated with focal cortical subarachnoid hemorrhage or with focal cortical superficial siderosis. They may also be seen in patients with CAA-related lobar hemorrhage, microhemorrhage or leukoencephalopathy. Migraine prophylactic agents such as verapamil and topiramate may be useful in stopping frequent recurrent TFNEs. TFNEs are an under-recognized cause of apparent TIAs. It is important to keep TFNEs in the differential diagnosis when a patient presents with a presumed TIA as thrombolysis or anticoagulation is relatively contraindicated in CAA. Gradient echo MRI should be performed to exclude microhemorrhages when TFNEs are suspected.

Bernard Galea, M.D.
University of Malta
Msida, Malta

Anna Pullicino
Malta Medical School
Msida, Malta

Patrick Pullicino* M.D., Ph.D.
Kent and Canterbury Hospital,
Canterbury, UK
P.Pullicino@kent.ac.uk

**Corresponding Author*

Clinicians most frequently associate cerebral amyloid angiopathy (CAA) with intracerebral hemorrhage or with a clinical picture of vascular cognitive impairment.[1] There have however, been increasing clinical reports documenting that CAA may cause a variety of acute clinical neurological manifestations.[2] Although these phenomena are superficially similar to TIAs and may be mistaken for them, they have clinical time profiles and progressions that can distinguish them from TIAs clinically. They appear to be caused by different manifestations of the complications of CAA and are now known as transient focal neurological episodes (TFNE).[2-3]

CAA frequency increases with age with approximately 50 % of individuals over the age of 75 being affected. The exact cause of CAA remains uncertain however increased production and/or decreased breakdown of amyloid proteins may have a role. CAA predominantly affects occipital regions of the brain followed by frontal and temporal areas. Cerebellar vessels are less commonly affected.[3] The Boston criteria is the current standard criteria for diagnosis of CAA. In this review, we attempt to classify and describe the different causes of TFNE's in CAA.

Clinical Manifestations of TFNEs

TFNE's include all neurological phenomena that may be otherwise attributed to TIA's as well as other atypical symptoms that are specific to TNFE's.[1] The duration of TFNE's is quite similar to TIA's with most symptoms resolving before 24 hours. Some cases of TFNE's have been reported to occur for longer but resolve eventually unlike major cerebrovascular accidents. Symptoms similar to those of TIA's include sensory disturbances, motor weakness, visual disturbances and language impairment. Atypical symptoms include associated headache and focal jerking [2-3]

TFNE's vary from TIA's in that symptoms often spread to contiguous body parts much like migraine attacks.[3] TFNE's also have a more gradual

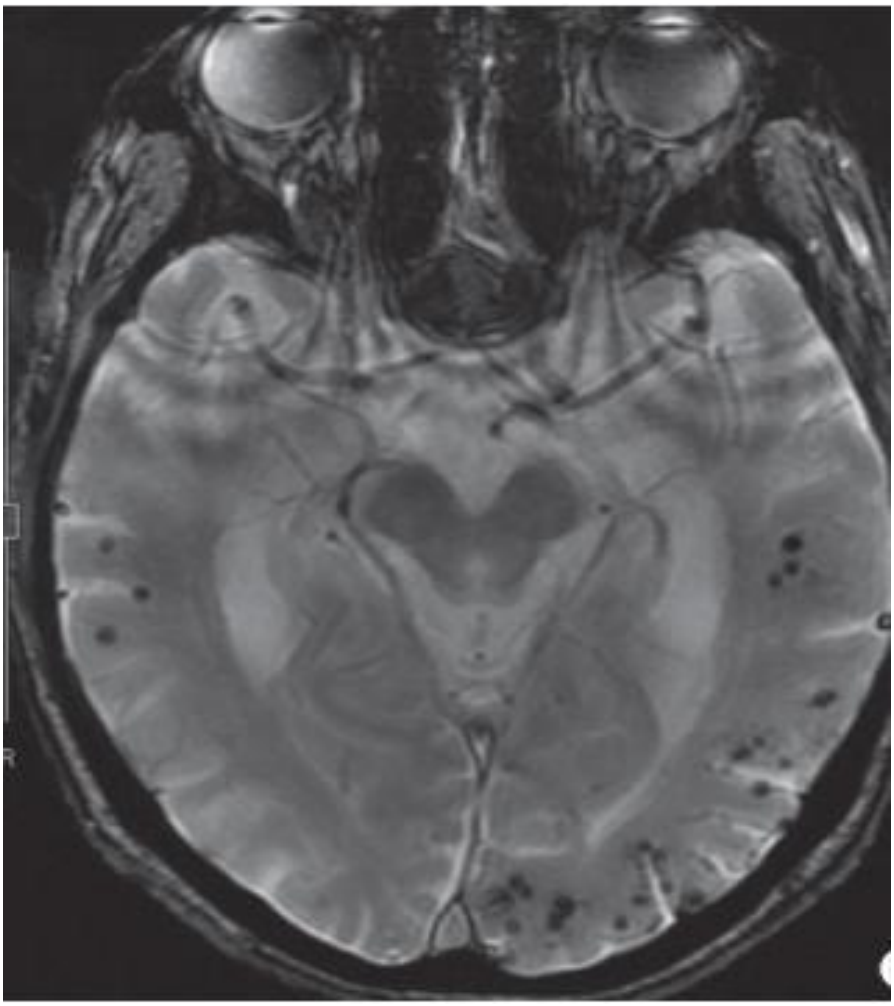
onset unlike TIA's which often have an abrupt onset. TFNE's are also more likely to occur multiple times during the day unlike TIA's which usually happen in single episodes or if multiple usually days apart.[2,3] TFNEs may occur repeatedly over several months and be a very intrusive symptom for the patient.[25] The typical presentation of a TFNE is of parasthesias involving one or more limbs that spread gradually to involve contiguous areas of that same limb. A TFNE may resolve partially or completely before recurring again later on during the day.

TFNEs with Cerebral Microhemorrhage.

Cerebral microhemorrhage that occur as part of CAA (Figure 1) may be a cause of TFNE's.[10, 18] Microhemorrhages are visualised in 47.4% of patients with CAA confirmed on pathology.

Risk for eventual conversion to lobar intracerebral hemorrhage (ICH) is significantly increased at the sites of microhemorrhage. The risk for microhemorrhage is higher in local areas of confirmed CAA and is directly proportional to the density of amyloid deposition in blood vessels. Increasing numbers of microhemorrhages in a localised area may be suggestive of a future ICH so this can be used as a predictive tool. While most cerebral microhemorrhages are usually asymptomatic, microhemorrhages were associated with TFNE's in several case studies.[6,11-14]

Figure 1: *Multiple bilateral microhemorrhages in a lobar distribution* [12]



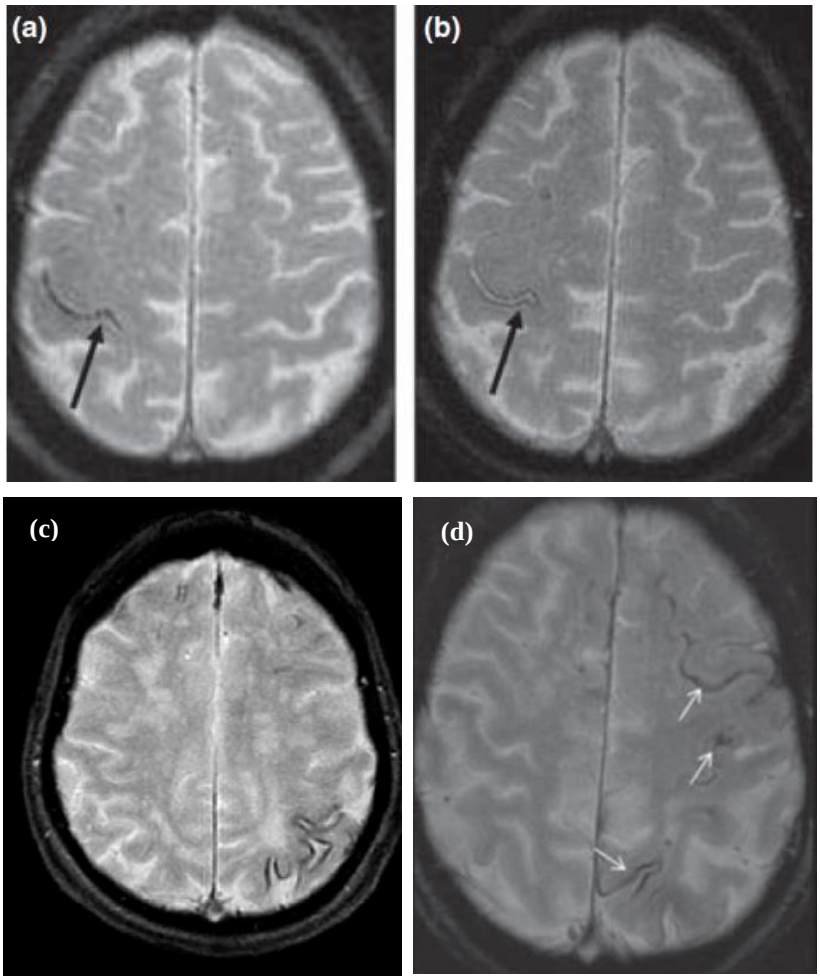
TFNEs with Focal Cortical Subarachnoid Hemorrhage and Cortical Superficial Siderosis.

The most frequent cause for TFNE's in CAA is focal cortical subarachnoid hemorrhage with resulting Cortical Superficial Siderosis (CSS).[16] (Figure 2) In CAA, subarachnoid hemorrhages typically occur in a focal convexity pattern along brain areas with a high CAA density. [4] Eventual conversion to superficial siderosis occurs after blood products are cleared from CSF spaces. [5] CSS can be defined as hemosiderin deposits in the subpial space that occur after repeated bleeding into the subarachnoid space. CAA-mediated subarachnoid hemorrhage may present with TIA-like symptoms as well as headache and ictal symptoms.[8-9]

CSS occurs in up to 61% of pathologically confirmed cases of CAA and increases the risk for lobar ICH significantly.[16,17] Even though CSS occurs mainly in areas where previous microhemorrhages were present, it generally occurs in patients with CAA with a lower microhemorrhage overall burden.[4] CSS associated with CAA may also be found in Alzheimer's Dementia and/or mild cognitive impairment patients who have a higher prevelance of CSS when compared to the general population. Current Boston Criteria for diagnosis of CAA does not include subarachnoid hemorrhage.[18-19]

15 % of patients with CAA experienced TFNE's at some point in one multicenter cohort study. Patients with CAA and evidence of CSS were more likely to experience TFNE's when compared to CAA patients with no evidence of subarachnoid hemorrhage or CSS (50% vs 19%). 50 % of TFNE patients experienced subsequent ICH over a median period of 14 months.[1,2]

Figure 2: *a) Right rolandic sulcus subarachnoid hemorrhage on T2-Gradient Echo MRI b) Conversion of blood to CSS 8 months later[8] c) Cortical Superficial Siderosis (CSS) left parietal lobe on T2- Gradient echo in a patient presenting with a transient aphasia and right motor deficit. [1] Also shows bilateral white matter T2 hyperintensity due to leukoencephalopathy. d) CSS in multiple gyri (arrows) in a patient with recurrent spreading parasthesia in right upper limb.[15]*



TFNEs Associated with Intracerebral Hemorrhage.

ICH is known complication of CAA.(Figure 3) The Helsinki ICH study found that 20% of ICHs occurred in patients with CAA in cortical areas that had higher amyloid angiopathy volume.[22]

ICH in CAA patients typically follows a lobar distribution rather than affecting deep structures of the brain as amyloid is deposited preferentially in meningeal and cortical blood vessels. Even though a significant proportion of ICH symptoms never resolve, CAA-mediated ICH has been implicated as a cause for TFNE's with complete resolution of symptoms in these instances. [21-24]

TFNEs with Cerebral amyloid angiopathy-related inflammation

Apart from hemorrhagic events, CAA is also associated with transient focal white matter vasogenic edema.(Figure 4) These white matter changes exert a variable degree of mass effect but are not enhanced by contrast. These lesions tend to present with worsening cognition, headache and stroke like symptoms. They may give symptoms spreading gradually to contiguous body parts. These lesions usually present with subacute cognitive changes, seizures and headache. Symptoms are usually transient in nature but they tend to last longer than TFNE's. These lesions may respond to steroids, or immunotherapy.

Figure 3: *Intracerebral hemorrhage in a patient with CAA confirmed using Boston criteria. (two separate axial planes from same image)* [18]

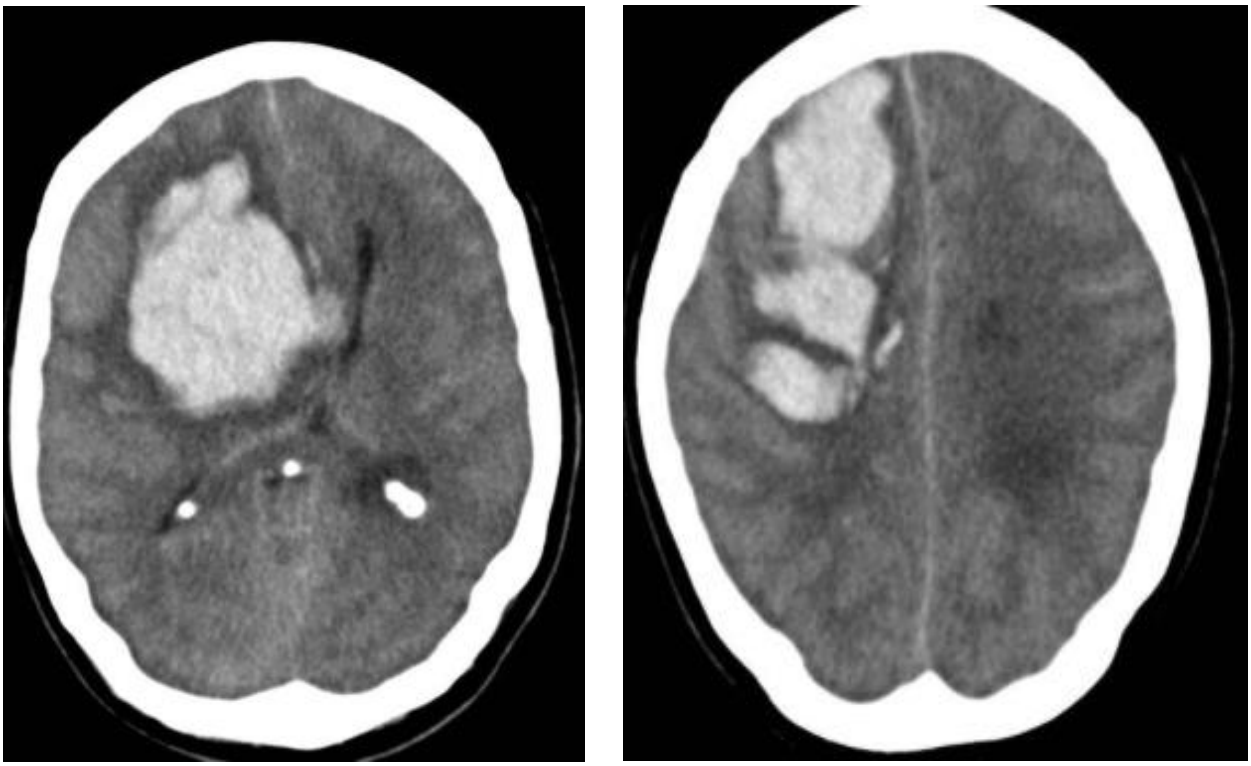
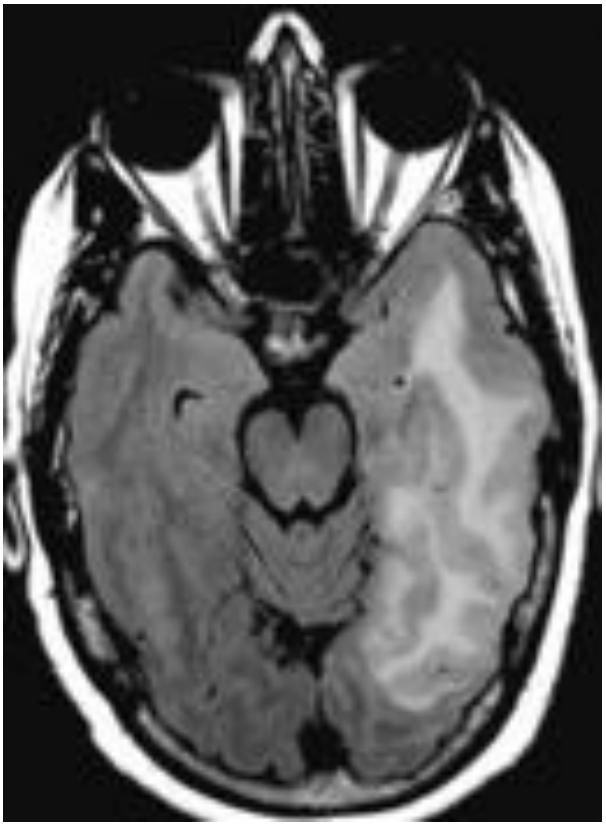


***Figure 4**: Cerebral amyloid angiopathy-related inflammation involving left temporal and occipital lobes.*[26]



TFNEs with leukoencephalopathy

Leukoencephalopathy (Figure 2c) volume is directly related to CAA volume and tends to occur predominately in the frontal and parietal lobes first (70 % of all patients) followed by temporal and occipital regions (15% and 10 %).[25,26] Leukoencephalopathy is associated with an increased risk of subsequent ICH in the areas most heavily affected. This finding suggests that ICH in CAA may occur as a result of two separate but related complications of CAA (dysregulated blood flow and increased susceptibly of hemorrhage from blood vessels with amyloid deposits).[25,26]

Several TIA-like episodes in known CAA patients had no evidence of overt pathology on imaging (subarachnoid hemorrhage, CSS or ICH) but did have a variable degree of leukoencephalopathy. This finding suggests that focal ischemia related to these white matter changes are another cause for TFNE's in CAA.[7,25,26]

Conclusion

CAA mediated TFNE's are an under-recognized cause of acute neurological symptoms. It is important to keep TFNEs as part of the differential diagnosis when a patient presents with a presumed TIA. The features distinguishing TIA's from TFNE's should always be sought using appropriate history taking, clinical examination and specific imaging.

Thrombolysis using IV-tPA or oral anticoagulation has been reported in patients with CAA-related TFNE's due to the misdiagnosis with TIA. [5, 20] Patients with TFNE's treated with thrombolytic therapy or anticoagulation were more likely to develop ICH when compared to those who did not receive therapy.[6] This finding questions the safety of the usage of anticoagulation / thrombolytic therapy in patients with TIA-like symptoms without prior exclusion of CAA-mediated subarachnoid hemorrhage using specific blood sensitive imaging such as T2-gradient echo MRI.[5,6,20]

Recent case reports suggest that agents used in migraine prophylaxis (verapamil or topirimate) may have a role in treating frequent recurrent TFNE's that persist over weeks or months and do not resolve spotaneously. In TFNE's caused by cerebral amyloid angiopathy-related inflammation, the lesions may respond to steroids.

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Review Article

Pollution and Cardiovascular Health in Malta – A Review

Jeremy Fleri-Soler, Andrew J Cassar-Maempel

Introduction

The leading cause of global mortality is cardiovascular disease (CVD) and in 2016 alone over 17 million premature deaths were attributed to CVD.[1] Whilst previously there was only weak evidence to suggest an association of air pollution as a risk factor for CVD,[2] in recent years, the impact of air pollution has emerged as an independent, strong and modifiable risk factor for CVD. The importance of air pollution on CVD is now being considered above other more traditional factors such as high cholesterol and reduced physical activity.[3]

Exposure to fine particulate matter has been shown to increase the risk of acute coronary syndromes, yet more concerning are the studies which have shown the greater extent of its effects over a longer period of time, reducing life-expectancy by a number of years and being responsible for 19% of cardiovascular mortality (>3 million deaths)[1]

The purpose of this review is to identify significant studies investigating air pollution and its effects on cardiovascular health while also considering our situation in Malta, as a small, highly populated country.

Jeremy Fleri-Soler MD, MRCP (UK)
Higher Specialist Trainee in Cardiology,
Mater Dei Hospital
Msida, Malta

Andrew J Cassar-Maempel* MD, MRCP (UK), FACC, FSCAI, FESC
Consultant Cardiologist
Mater Dei Hospital
Msida, Malta
andrew-j.cassar-maempel@gov.mt

**Corresponding Author*

Epidemiology – prevalence / impact

Air pollution is a worldwide problem with almost all people exposed to some degree.[1] When discussing health care, the term air pollution encompasses gaseous pollutants present at ground level (ozone, carbon monoxide (CO), nitrous dioxide (NO_2) and sulfur dioxide (SO_2)) as well as airborne particulate matter (PM), all of which are able to enter the body through the airways. The latter is subdivided into three categories according to size of particles: coarse matter (<10μm - ≥2.5 μm), fine matter (<2.5 μm - ≥0.1μm) and ultrafine particles (<0.1 μm).[3]

Primary pollutants, such as soot particles, nitrous dioxide and sulphur dioxide, are emitted directly into the air by combustion of fossil fuels. Secondary pollutants are formed in the atmosphere from other components, such as ozone which is formed through complex photochemical reactions of nitrogen oxides and volatile organic components. Sources of coarse matter (PM_{10}) include dust, soil and dirt physically thrown into the air by wind or movement of vehicles. Once airborne, PM_{10} may be inhaled and deposited into the throat and upper airways. On the other hand fine matter ($PM_{2.5}$) is mostly found in smoke and haze and is attributable to combustion processes taking place in human industry, power plants, motorized vehicles and residential heating using oil, coal, or wood. $PM_{2.5}$ is of particular health concern as it is able to travel deeply into the respiratory tract and into the small airways.[4] It is also important to highlight that $PM_{2.5}$ can travel over long distances (>100km) imposing its effect over a wider area.[3]

There is major variability in the levels of air pollution globally. As expected, lower-income countries have higher household pollution due to consumption of solid fuels for domestic heating and cooking. However this is also a problem in the west where despite spending most of their lives indoors, people are still exposed to $PM_{2.5}$ which has been shown to infiltrate buildings. More concerning is a shift to using 'environmentally-friendly' biomass

fuels for heating over traditional electrical means, particularly prevalent in Northern Europe, creating major indoor air quality problems.[3] Large urban-rural differences are found for soot (average 38% higher), NO_2 (63% higher), and ultrafine particle numbers. Temporal variation of daily air pollution concentrations is also present. For example, ozone concentrations are highest during the warmest, high-intensity sunlight hours of the day; traffic-related pollutants, such as ultrafine particles and soot, often peak during the morning and evening rush hours resulting in high exposures for people commuting. Wind direction, wind speed and atmospheric stability also effect daily pollution levels.

Air Pollution and Mortality

Cardiovascular Mortality

In 2004 the American Heart Association (AHA) first released a statement on their position of the effect on air pollution on health, concluding that exposure to particulate matter is indeed an adverse factor in cardiovascular morbidity and mortality.[5] In a further review in 2010 the AHA consolidated their position by claiming exposure to $PM_{2.5}$ in the short term may trigger cardiovascular events but that there is an even greater effect in long-term exposure, reducing life expectancy by a few years.[5] One meta-analysis found a pooled effect of a 6% increased risk per 10 $\mu g/m^3$ increase in $PM_{2.5}$ in all-cause mortality and as much as an 11% per 10 $\mu g/m^3$ $PM_{2.5}$ increased risk in cardiovascular mortality, which was higher when compared to death from non-malignant respiratory disease.[6]

In recent years, the European Study of Cohorts for Air Pollution Effects (ESCAPE study) was launched with an aim to investigate the effect of pollution on the health of >300,000 individuals across Europe. They used standardized models for measurements of exposure and subsequent health effects whilst trying to eliminate confounding factors such as race and social status that may exist between regions.[7] ESCAPE revealed a statistically significant effect of $PM_{2.5}$ on all-cause mortality, with a non-significant association with cardiovascular mortality specifically. The effect was exerted with mean $PM_{2.5}$ levels lower than those currently recommended, a trend that will be found across many studies.[8]

Cardiovascular Disease

Cardiovascular effects of air pollution are now well-recognized and studies have increasingly shown pollution to both exacerbate existing heart conditions as well as predispose the individual to the development of cardiovascular disease including coronary artery disease, cerebrovascular disease and peripheral artery disease.[3]

Coronary artery disease

Long-term exposure to $PM_{2.5}$ was associated with an increased risk of coronary artery disease in previously healthy, post-menopausal women. In the Women's Health Initiative Study, exposure to >65,000 women over 4 years in 36 different cities showed that risk of cardiovascular events was increased with increase in air pollution exposure. There was a hazard ratio of 1.21 for developing a first coronary event and a hazard ratio of 2.21 to die of the coronary event for every 10$\mu g/m^3$ increase in $PM_{2.5}$.[9]

The ESCAPE study involved more than 100 000 participants without a previous history of cardiovascular disease from 11 cohorts across Europe over the period 1997-2007. It found a 12% increased risk of coronary events per 10 $\mu g/m^3$ in PM_{10} and a 13% increased risk of coronary events per 5 $\mu g/m^3$ increase in $PM_{2.5}$. Most importantly, positive associations were also observed below the current recommended annual European limit for $PM_{2.5}$ and PM_{10}.[10-11] Both of these studies found a higher risk in people over 60 years of age and non-smokers, with $PM_{2.5}$ exerting its effect at less than 25$\mu g/m^3$, the current legal limit recommended. These findings suggest pushing for the lowering of European limits of air pollution so as to better protect the public from cardiovascular disease.

Another study investigated the increased risk of myocardial ischemia with exposure to $PM_{2.5}$ in patients known to suffer from ischemic heart disease, through repeated exercise stress testing. There was an increased risk of myocardial ischemia (as defined by ST-segment depression on exercise stress testing) in those people who had been exposed to higher levels of $PM_{2.5}$ or the gaseous pollutants NO_2 and CO.[12]

More recently, a study involving six metropolitan areas in the United States, showed an association between air pollution and the development / progression of coronary artery calcium over 10 years, with a particular stronger

association in people aged over 65, the hypertensive and non-obese. Also of interest was that the mean $PM_{2.5}$ level in these areas was lower and acceptable by international standards.[2]

Cerebrovascular disease

The Women's Health Initiative study reported a 35% increased risk of stroke and death from cerebrovascular disease and 83% increased risk of death from cerebrovascular disease per 10μg/m^3 increase in long-termPM2.5 exposure.[9]

The ESCAPE study found a 19% increased risk of stroke per 5 mg/m3 increase in PM2.5 in nearly 100 000 participants from 11 cohorts across Europe.[10]

Peripheral artery disease

Furthermore, air pollution and living closer to a main road was also been associated with peripheral artery disease as defined by worse ankle brachial indexes.[13]

Heart failure

While much research has suggested a link between coronary artery disease and air pollution, studies into its possible association with heart failure in the long-term are still unsubstantial. One meta-analysis included 35 studies from across North and South America, Europe and Asia looking into more than 4 million events. It linked an increased risk of hospitalization or mortality due to heart failure with increased levels of both CO (per 1ppm), SO_2 (per 10ppb), NO_2 (per 10ppb), $PM_{2.5}$ (per 10μg/m3) and PM_{10} (per 10μg/m3), as much as 3.5%, 2.4%, 1.7%, 2.1% and 1.6% respectively.[14]

Arrhythmias and arrest

The effect on ventricular arrhythmias is unclear, with studies providing inconsistent evidence. While studies investigating a clear relationship between arrhythmias and pollution by measuring the rate of activation of implantable cardio-defibrillators have so far come up short, (15)other studies have reported an association between sudden cardiac death and out-of-hospital arrest with pollution.[16]

Pathophysiology

Although recent data has shown a strong influence of air pollution on cardiovascular disease, the exact mechanism by which air pollution increases the risk of cardiovascular problems remains unclear. Several hypothesis of pathophysiology have been suggested.[3]

Vascular Dysfunction

One proposed mechanism is that exposure to air pollution predisposes to vascular dysfunction with an increase in vascular tone and thus blood pressure, contributing to long-term sequelae. A study discovered that short-term inhalation of $PM_{2.5}$ and ozone contributed to an acute rise in mean arterial blood pressure, secondary to acute arterial vasoconstriction. Subjects had been exposed to concentration levels comparable to the urban environment.[17] Meanwhile larger observational studies have shown that exposure to $PM_{2.5}$ has led to persistent vascular dysfunction, and the lowering of $PM_{2.5}$ concentration exposure was associated with a drop in the mean blood pressure and a reduction in cardiovascular events.[18]

Atherosclerosis

Several studies have showed the association of formation of atherosclerosis with exposure to air pollution, in particular $PM_{2.5}$.[7] Using CIMT as marker for subclinical atherosclerosis, the Multi-Ethnic Study of Atherosclerosis (MESA) examined the progression of CIMT in comparison with $PM_{2.5}$ levels. Despite a mean follow-up of only 2.5 years, it showed a positive but not significant association between pollution and CIMT and a greater reduction on $PM_{2.5}$ levels was associated with a slower rate of CIMT progression.[19]

A cross-sectional meta-analysis of data from studies, including the previously mentioned ESCAPE, showed a 0.78% increase in CIMT per 5μg/m^3 increase in $PM_{2.5}$ as well as showing a positive association between CIMT and living proximity to high vehicular traffic.[7]

Increased Thrombogenicity

One hypothesis for the association of short-term exposure to $PM_{2.5}$ with acute cardiovascular events is the suggestion that prolonged exposure to air pollution results in endothelial damage and an increased likelihood of thrombosis through chronic inflammation and transient increases in plasma viscosity.[20]

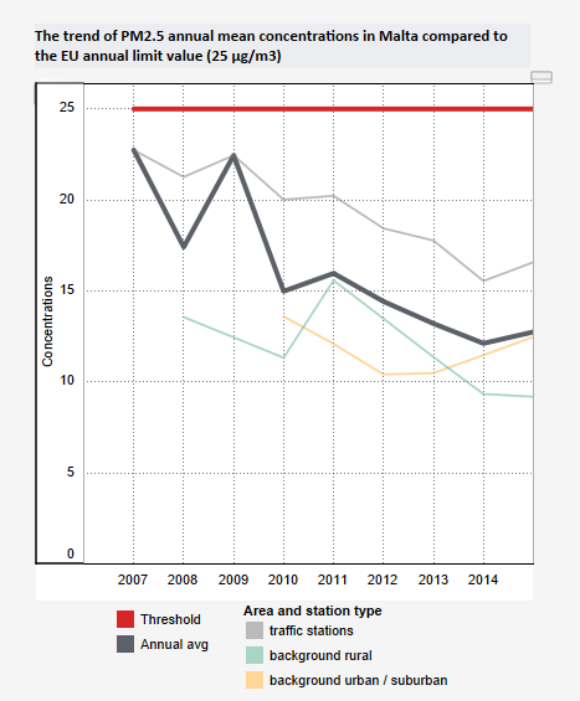
This effect seems to be particularly increased in patients who suffer from diabetes. A study confirmed this by investigating platelet function in

adults with diabetes. The study concluded that a relative increase in $PM_{2.5}$ was associated with a greater prothrombotic tendency of platelets as well as an increase in blood leukocyte number that was used as a marker for inflammation.[21]

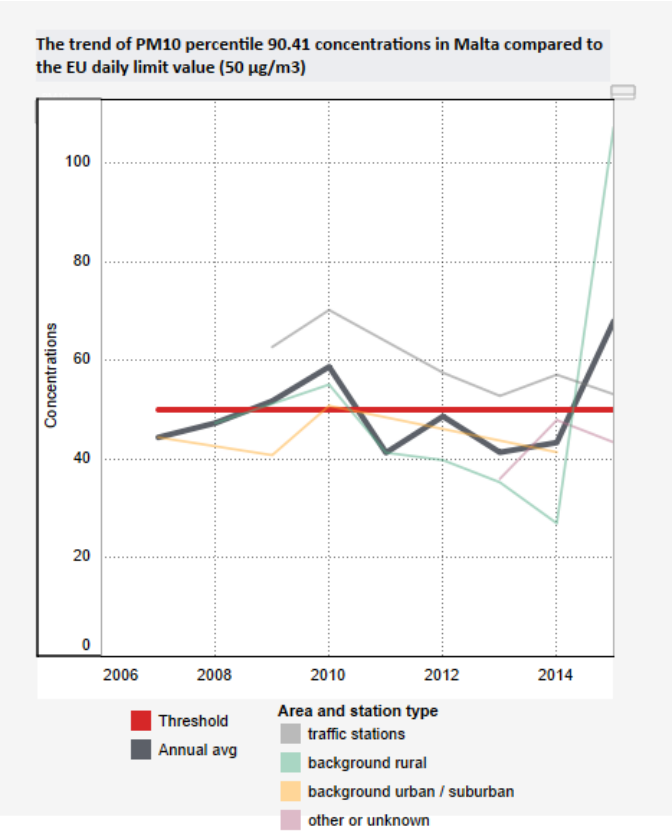
Air Quality in Malta

In recent years the European Union (EU) has recognized the negative impact that air pollution has on European health and economy and has set air quality limits which are regularly exceeded, especially in urban areas, the most troublesome pollutants being PM and ground-level NO_2.[22] Since joining the EU, Malta has committed itself to monitoring pollution levels and striving to reach targets agreed upon. The European Environmental Agency (EEA) monitors air pollution levels in all EU-28 member states through air quality data, which are submitted by each country to the EEA. The most recently published data encompasses the years of 2011-2015.[22]

As depicted below in Figure 1, taken from of the EEA report published in 2017, Malta has managed to remain below the EU's $PM_{2.5}$ limit of 25µg/m^3 with a mean of 10-20µg/m^3. The main contribution of $PM_{2.5}$ in Malta is identified as road traffic and energy production, with road traffic accounting for approximately 6 times of emissions when compared to energy production in 2013. More worryingly, emissions from traffic are on the upward trend.[22]



Data regarding PM_{10} in Malta can be found in Figure 2. The daily limit of 50µg/m^3 is regularly exceeded, peaking at a mean of 70µg/m^3 in 2015.[22] However no data was available in tracking sources of emissions.



Using this data, the EEA has been able to quantify the effect of air pollution and express it as premature deaths. Malta, with a population of 425,000 people at the time of the study, was found to have an annual mean $PM_{2.5}$ of 12µg/m^3. By extrapolation this was calculated to contribute to 220 premature deaths annually.[22]

The EU recommends annual mean levels of $PM_{2.5}$ and PM_{10} of 25µg/m^3 and 40µg/m^3 respectively, however no standards have been set for the chemical composition of PM and no standards set for $PM_{0.1}$ levels, the so-called "ultrafine particles".[3]

Although Malta reached $PM_{2.5}$ levels as recommended by the EU, it is important to also mention that in 2005 the World Health Organization (WHO) published global guidelines for air quality standards, in which the $PM_{2.5}$ limit was recommended at 10µg/m^3 as an annual mean and the PM_{10} limit was recommended at 20µg/m^3. Therefore at $PM_{2.5}$ of 12µg/m^3 Malta exceeds this.[23] In fact it is estimated that one third of Europeans

live in substandard air quality according to EU guidelines however as much as 90% are exposed harmful levels according to WHO criteria.[3]

A Timely Intervention

Since the harmful effects of air pollution have been clearly defined, the issue turns to the value of intervention on air quality control. A study from the United States investigated changes in life expectancy associated with changes in $PM_{2.5}$ levels during the 1980s and 1990s. A significant increase in mean life expectancy by 0.61 years was associated with a reduction of $10\mu g/m^3$ in $PM_{2.5}$ concentration, accounting for almost 15% of overall increase in life expectancy in areas studied.[24]

Another study used the ban on coal sales in Dublin in 1990 as a pivotal point in examining air quality, investigating mortality rates over the 6 years prior and comparing to the following 6 years. Average black smoke concentrations fell by $35\mu g/m^3$ and, in conjunction, non-trauma mortality decreased by 5.7% and cardiovascular deaths fell significantly by 10.3%.[25]

Strategy Propositions

In 2017 the *Lancet* Commission on Pollution and Health was published, which not only highlighted the effect of pollution on health but also proposed that global pollution can be controlled and pollution-related health problems prevented, advocating for several interventions. As Table 1 summarizes, such interventions are needed at population and individual level and as healthcare professionals we have a role to play too.

***Table 1**: Summary of Proposed Interventions at Different Levels of Society*[1,26]

	Government	Individual	Healthcare Professionals
Determine Exposure & Health Risks	• Scientific monitoring of population exposure • Funding research in adverse effects on health	• Increase awareness of susceptibility to exposure	• Promote research in public health, environmental science
Reduce Emissions at Source	• Regulations and enforcement towards reduction of emissions • Develop renewable energy	• Use of clean burning fuels • Use of active / public transport	• Using health economics to demonstrate to policy makers the long-term financial benefit of pollution reduction
Reduce Exposures Downstream	• Incentivize cleaner industry through use of air filters • Identify and intervene at exposed sub-populations	• Identify and avoid potential sources of exposure to self • Installation of home ventilation systems	• Public education in risks of exposure, encouraging behavioural change

Pollution control strategies are dependent on a determined government and an engaged, informed and empowered civil society, with necessary collaboration between different government agencies and non-governmental organizations (NGO's) both nationally and internationally. Successful intervention relies on primary prevention by eliminating pollution at the source, and an efficient system able to control pollution once present in the environment. Such proposals do not come into fruition overnight and therefore it is important that ambitious but attainable targets are devised, guided by both national and supranational standards such as WHO or the EU. For interventions to be effective they must have public support and be prioritized according to health effects, environmental damage and cost-effectiveness. Once implemented it is important to establish systems for monitoring the effects of such interventions and seek improvement in their significance.[26]

Examples of high impact interventions to reduce air pollution are the energy and transport sectors. Highly relevant to Malta, the recent shift of energy production from coal power plants to cleaner gas plants while also incentivizing industry to move towards cleaner, more efficient production technologies is a step in the right direction being taken along with many developed countries. An even better strategy would be funding research and converting to low-polluting energy production such as wind and solar plants which would not only improve cardiovascular health of the population but also reduce greenhouse gases and increase the economic efficiency of energy generation.

Transportation also carries a heavy impact on air pollution, significantly so with the Maltese population who are so reliant on personal transportation. Upgrading public transportation to be more efficient and affordable for the populous as well as restricting motorized vehicles from city centres and encouraging active transport with the creation of appropriate walkways and cycle-lanes will significantly improve air-quality.[26] Moving toward electric cars rather than diesel / petrol will be an inevitable step in the future which should rather be done sooner than later. Paris have already targeted a ban on non-electric cars by 2030 and Copenhagen recently announced a plan for a total ban on diesel cars by 2019.

On an individual level, one can limit the effect of air pollution on health by personally reducing contribution to pollution through reduction of energy or transport use as well as the use of clean fuels in energy consumption. In preventing personal exposure to pollution an increased awareness of susceptibility to pollution is needed with subsequent behavioral change to reduce exposure.[1]

Finally, as health professionals, we can contribute by emphasizing the relationship between pollution and adverse health effects, contributing research in environmental science, public health and health economics and supporting education in environmental health science in the younger generations.[26]

Conclusion

In recent years, the problem of air pollution and its harmful effects have been highlighted with ample evidence published supporting the significant association of air pollution with risk of adverse cardiovascular effects. As a nation, Malta has elevated levels of air pollution and trends towards further worsening in recent years as industry and economy are booming. This is most likely resulting in significant morbidity and mortality due to cardiovascular disease to the Maltese people. We must now turn to solution through determination of the population including taking care of our own roles as individuals in society, by pressuring policy makers and supporting interventions carried out on a national scale so as to reverse the trend.

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Review Article

Air Pollution and Inflammatory Bowel Disease

Ryan Falzon, Pierre Ellul

Abstract

The exact mechanisms through which IBD occurs are currently not known. There are several genetic and environmental factors that are implicated. What is known is that the incidence of IBD is commoner in Industrialised countries and in countries which are becoming more industrialised, the incidence of IBD is increasing. Pollution is one of the environmental factors that could be implicated in the increase in its incidence. In this review article we analyse the effects of pollution on the gut and the studies which try and shed light on the association between IBD and air pollution.

Key words

air; pollution; particulate matter; Crohn`s Disease; Ulcerative colitis; Inflammatory bowel disease

Ryan Falzon MD*
ryan.falzon@gov.mt

Pierre Ellul MD PhD FRCP MSc

**Corresponding Author*

Introduction

The incidence of inflammatory bowel disease (IBD),which consist of Crohn's disease (CD), ulcerative colitis (UC) and IBD unclassified (IBDU) is subject to considerable variation worldwide. IBD is commoner in industrialized countries. Recent data on the overall incidence rate ratios in all Western European centres was 1.9 (95% CI 1.5 to 2.4) for CD and 2.1 (95% CI 1.8 to 2.6) for UC when compared to Eastern European centres. The median crude annual incidence rates per 100,000 in 2010, for CD were 6.5 (range 0-10.7) in Western European Centres, compared to 3.1 (range0.4-11.5) in Eastern European Centres. For UC, the crude annual incidence rates were 10.8 (range 2.9–31.5), in Western European Centres compared to 4.1(range 2.4–10.3). Similar data was also present for IBDU.[1]

Although, over the last decade, the discovery of genes linked to susceptibility to IBD was thought to be a major breakthrough, current studies demonstrate that that this only explains approximately 20-25% of the hereditary variance. Thus, environmental factors are likely to contribute significantly to disease pathogenesis.[2]

Furthermore, currently the role of microbiome in IBD is being investigated world-wide. IBD is linked to changes in the composition of intestinal microbiota. It was noted that there is an increased proportions of Anctinobacteria and Proteobacteria, together with a decrease in the diversity and proportions of Firmicutes. Furthermore, the microbiota of patients in remission was found to be different than that of active IBD patients.[3]

The fact that the incidence of IBD increased rapidly during the past decades and that the increase has been particular in several developing countries, where the incidence of IBD was considered to be low, led to the suggestion of the importance of environmental factors, one of them being pollution.[4]

Environmental factors and IBD

Exposure to cigarette smoking is the best characterised among the environmental factors potentially contributing to CD, but other

environmental factors such as lack of exposure to pets and gardens as well as living in an urban area are also associated with an increased risk.[5]

Multiple sclerosis shares features with IBD, including the role of environmental, microbial factors and genetic factors. With regards to environmental factors both diseases are possibly associated with poor air quality.[6] Currently, no study has directly assessed how genes and air pollution interact in IBD. This is mostly in view of the difficulty to quantify the contribution of air pollution and IBD susceptibility. However, smoking and common IBD-related genes have been evaluated in this setting.[7]

Theoretically, there is a good rationale for gene-environment interactions in these patients. [8] This is due to the fact that may genes associated with IBD are involved in bacterial recognition and handling, and air pollution may modulate this effect. This is well demonstrated in patients with polymorphisms in NOD2 and autophagy genes which possibly enhance the effects of pollution.[9]

Although studies are limited with regards to the role of air pollution as a risk factor for IBD, studies of the effect on cardiorespiratory system have shown that there would be an increase in the circulating inflammatory cytokines, such as tumour necrotising factor alpha[10] and leukocytes.[11]

Ambient air quality had been associated with flares in multiple sclerosis, a disease which shares pathogenic mechanisms with IBD. In a study carried out by Oikonen *et al*. the relationship between the occurrence of multiple relapses and ambient air quality was observed. The results were that the odds of relapse were 4-folds higher during months with highest quartile of airborne particulate matter. In view of these results the authors stated that poor ambient air quality may lead to increased susceptibility to communicable infections or augment already existing peripheral inflammation leading to disease flare.[5]

Therefore, this study shed light on the possibility a similar systemic inflammatory response to poor air quality exposure may play a role in IBD flares, leading to increased hospitalisations.[12-13]

Components of Air pollution

Air pollution is a mixture of chemicals which are well known to cause adverse health effects. This heterogeneous mixture of substances commonly consists of gases such as sulphur dioxide, nitric oxide, nitrogen dioxide, carbon monoxide, carbon dioxide and ozone, volatile organic compounds and particulate matter.[15] The components of air pollution depends mainly on the source of the pollution itself ranging from fuel combustion such as home furnaces and vehicles to livestock 'emissions'.[16] Therefore, depending on where a person lives, travels and work; s/he is exposed to a unique mixture of pollutants.

Most of the attention has been given to ozone and particulate matter (PM). Ozone has been linked with induction of airway inflammation and damage, resulting in increased cell permeability and breakdown of tight junction integrity, and a similar process may take place in IBD.[17] PM leads to a number of effects. Nitrogen dioxide is linked with adverse health outcomes.[18] PM is defined according to the aerodynamic diameter, being divided into either fine particles, these being smaller than 2.5μm or coarse particles, the latter having a diameter which is more than 2.5μm but less than 10μm.[19]

Other components of air pollution may be linked with health effects, however only selective ones are used as surrogate markers of quality of air. Therefore such components may be missed due to lack of data.[20]

Pollution exposure to gut mucosa

The gastrointestinal tract is exposed to high concentration of pollutant PM, as they are quickly cleared from the lungs by the muco-ciliary system and transported to the gut. Another source of intake is the oral route due to contaminated food and water supply. It is estimated that 10^{12}-10^{14} particles are ingested per day.[19]

Current treatment strategies, of using thiopurines, methotrexate and anti-TNF-alpha are targeted to reduce the effects of the immune system.[21] Thus dysregulation and over reactivity of intestinal immune responses are potential mechanisms for IBD.[22]

As demonstrated in various studies, air pollutants lead to a systemic immunomodulatory/ inflammatory effect, and therefore many autoimmune diseases are linked with urban living and industrialisation, as seen in the hygiene hypothesis.[23] This states that the decrease of infectious burden, in industrialised countries, has led to a rise in the incidence of autoimmune diseases. It was first proposed by Strachan in 1958. [24] Several epidemiological studies have investigated

this link. These have demonstrated that exposure to cowsheds and farming at a young age can prevent the development of atopic diseases, especially if this is during pregnancy.[25] However, up to date there is still little research that studies autoimmune disease and air pollution directly.[26] Most data about the possible mechanisms such as systemic oxidative stress and increased cytokine levels in the blood come from respiratory and cardiovascular research.[27-28]

Environmental effects on the Gut Microbiota

Microbiota plays an important key role in IBD, and therefore another effect of air pollution may be the disruption of this balance.[29]

Microbial imbalance reported in IBD may be a result of changes in the environment, including hygiene and food. This results in imbalance in microbial-host relationship leading to mucosal barrier dysfunction and a decrease in the microbiota diversity.[30] Dysbiosis leads to an increase in the pathological bacteria including *Mycobacterium avium paratuberculosis ssp (*MAP) and a decrease in beneficial bacteria such as Lactobacilli and Bifidobacteria.[31]

Several studies have highlighted the high prevalence of MAP in CD patients.[32] However, although a number of microbes have been associated with the disease, no single agent was identified. Many of the features of modern lifestyle have been linked with IBD including crowding, domestic hygiene and antibiotic usage in childhood.[33]

Geographical variation in IBD and the living environment

Northern Europe and North America have the highest incidence rates of IBD worldwide. This may indicate common aetiological factors. Studies demonstrate that in countries (e.g. South Korea, China, India, North Africa, French West Indies and Thailand) which are becoming more westernised, the incidence of IBD is increasing.

Within Europe, there are also marked differences in the rates of IBD between different countries. These differences may be due to difference in the cohorts, organisation of health care and methodology of data collection. However, in the European Collaborative Study on IBD, the rate difference between Northern and Southern Europe decreased and similarly there is a sharp increase in IBD diagnosis in Eastern European countries. Over the past two decades, these populations have 'westernised' their lifestyle, leading to increase in pollution. Immigration has also shown to leave an impact on the prevalence of IBD. It was shown that individuals who have emigrated to westernised countries and then returned back to their native country, demonstrated an increase an increase in developing IBD, especially UC, suggesting that environmental factors related to industrialisation may play an important factor.[34]

Relationship between smoking and pollution-relevance to IBD

Smoking is associated with the severity of CD; however it is inversely associated with UC, occurring more in non-smokers and ex-smokers. The similarities in exposure between air pollution and smoking, brings to relevance the possibility that air pollution may also have an important effects on the development of IBD. Some mechanism involved in mediating the intestinal effects of cigarette smoking might play a role on how air pollution affects the intestines.[4]

One obvious difference is that air pollution does not contain nicotine, which is found in cigarettes. Although nicotine appears to cause some of the clinically relevant effects, nicotine replacement alone does not seem to replicate smoking in terms of impact on IBD. [20] Particulate matter is the common component between air pollution (from other sources) and smoking.

Effects of Pollution on the Gut

In between the external luminal environment and the internal body proper, lies the highly regulated epithelial monolayer, which besides the function of absorption also provides important immune and barrier surveillance mechanisms. Mutlu *et al*. showed that with exposure to PM, there was a decrease in the epithelial barrier, associated with rearrangement of epithelial tight junction proteins. This is linked to the generation of free radical oxygen species (ROS). The increase in permeability has been associated with intestinal inflammation. This may be due to the fact that there is an influx of the PM and microbial products into the lamina propria, thus increasing interactions with immune cells.[19]

The microbial entry will induce an inflammatory response by dendritic cells and

macrophages leading to systemic inflammation, altering the luminal environment of the gut, thus allowing for growth of particular microbial strains. The latter would be suited to survive in an inflammatory environment. These changes may also lead to production of altered metabolites such as butyrate which further increases the intestinal permeability.[19]

In a study conducted in Singapore, it was demonstrated that elevated atmospheric PM was associated with greater circulating polymophonuclear leukocytes. Healthy men who were exposed to diesel fumes, showed an increase in the level of plasma cytokines, particularly TNF-alpha, which is an important mediator in IBD. Currently one of the common drugs used in the management of both CD and UC is anti-TNF-alpha medications. Furthermore, Miller et al. also showed an impairment of vascular function on exposure to diesel exhaust. Such studies may continue to explain the link between pollution and the disease.[13]

Epidemiological studies have been performed as to assess the association between pollution and IBD. A study carried out by Kaplan *et al*, concluded that there is no association between pollution and IBD epidemiologically, for newly diagnosed cases. However, an association was revealed in some subgroups. Individuals, under the age of 23 years of age were more likely to be diagnosed with CD if they lived in regions of higher pollution (OR 2.31), with a linear association between risk and increased air NO levels. PM exposure was mostly associated with CD (OR 1.73), while UC was associated with higher sulphur dioxide levels for individuals under 25 years of age (OR 2.0). However, middle aged adults, between the age of 44 and 57 years, diagnosed with Crohn's disease were less likely to live in regions with elevated concentration of Nitrogen Dioxide.[35] Therefore this may indicate that traffic-related pollutants, such as nitric oxide and industrial based pollutants such as sulphur dioxide may have age specific effects on the development of IBD.

In another study, data from the Wisconsin Hospital Association, from the year 2002 was used to identify IBD related hospitalisation. Data regarding average annual emissions density was obtained from the Environmental Protection Agency. This study demonstrates that there is an association between adult hospitalisation (81.3 hospitalizations/ 100,000 people per year) and total pollutant density.[36] There was a mean of 81.3 IBD hospitalizations/100,000 population per county (range 0-174). The total criteria pollutant emissions density correlated significantly with adult IBD hospitalizations (Pearson's correlation coefficient (rho) 0.28, p=0.020). On Poisson regression, a 1-log increase in the density of total criteria pollutant emission was associated with a 40% increase in the rate of IBD hospitalizations (incidence rate ratio [IRR] 1.40, 95% confidence interval [CI] 1.31-1.50) This was similar for both ulcerative colitis (UC) (IRR 1.48, 95% CI 1.27-1.73) and Crohn's disease (CD) hospitalizations (IRR 1.39, 95% CI 1.26-1.52). Analysis of each of the individual criteria pollutant emission densities revealed a significant association for all the component criteria pollutants.[37]

Studies had also shown that an urban household was associated with a higher incidence of IBD, in light of the fact that urban regions are associated with higher air pollution levels.[38-40] A systematic review and meta-analysis demonstrated a positive association between the urban environment and both CD and UC. However due to the heterogeneity in the study design and results, the temporal link between urban environment and the risk of IBD development could not be concluded with confidence.[41]

Although the relationship between ambient air pollution and IBD appears plausible from an epidemiologic stand point, one should interpret them with caution, due to possible bias. In such studies, mixed air pollution is observed, where one measured pollutant may also serve as a marker of other confounding exposures.[20]

There are multiple other studies which had examined the relationship between urban living and IBD. A study carried out in Manitoba showed that individuals who had ever lived on a farm, were less likely to develop CD (OR 0.62, 95 % CI 0.46–0.85, p=.003), while urban living was associated with higher risk of both CD (IRR 1.29, 95 % CI 1.17–4.41) and UC(IRR 1.12, 95 % CI 1.04–1.21). Likewise, rural patients in the UK were less likely to develop early-onset CD (OR 0.36, 95% CI 0.14-0.95) and UC (OR 0.66, 95% CI 0.51-0.85).[42]

Li X et al., using a nationwide database linking the Swedish Census to the hospital discharge register, studied the link between education level and occupation and hospitalisation for IBD. The data obtained was on all first

hospitalisations for UC and CD. The results show that there was a significant decrease in the standard incidence ratio for Crohn's disease in both men and women who had an educational level of more than 12 years. However, a significant increase in the standard incidence ratio was seen amongst drivers in all cohorts. The reason for this is unclear but the probable daily exposure to pollutants has to be actively considered.[43]

Finally, in laboratory studies, where mice were fed particles sieved from an urban air filtration system developed increased pain,[44] had raised pro-inflammatory IL-8 levels and exhibited dysbiosis.[38]

Conclusion

Several environmental factors have been linked with IBD, including ambient air pollution. Though it might not be a leading cause, it can definitely be a significant contributor. The underlying mechanism and the interaction of all these risk factors to cause IBD have not yet been elicited. A limitation of these studies is the different methodological methods associated with studying environmental risk factors in IBD.[4]

Although data is suggestive of this association especially epidemiologically, further efforts should be done to determine the effects of pollution on the biological mechanisms.[20]

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Review Article

Monoclonal gammopathy of significance

David Dingli

Abstract

The presence of a monoclonal protein in the serum and/or urine is a common clinical condition that increases in incidence with age. Although the diagnosis is most often monoclonal gammopathy of undetermined significance (MGUS) there are many conditions associated with the presence of a monoclonal protein in a patient. Several of these are potentially life-threatening and the presence of the M protein should serve as a clue to these diagnosis. The size of the monoclonal protein has very little bearing on the association with other disorders. This concise review highlights the better known associations between monoclonal proteins and other symptoms, making them monoclonal gammopathy of clinical significance.

David Dingli, MD, PhD
Division of Hematology
Department of Internal Medicine
Mayo Clinic
Rochester
dingli.david@mayo.edu

The concept of monoclonal gammopathy of undetermined significance (MGUS) was coined by Dr Robert A. Kyle in 1978 to describe a clinical state when a monoclonal protein is identified in a patient's serum or urine in the absence of evidence of end organ damage such as multiple myeloma, primary (AL) amyloidosis or Waldenstrom's macroglobulinemia.[1] The presence of a monoclonal protein by necessity infers the existence of clonal plasma cells in the bone marrow that produce the protein and for this reason, MGUS belongs to the spectrum of clonal plasma cell disorders. This group of conditions varies from the benign MGUS to the highly malignant primary plasma cell leukemia. The current diagnostic criteria for MGUS require that there are less than 10% clonal plasma cells in the bone marrow and the monoclonal protein concentration in the serum must be less than 30g/L (for IgG, IgA, and IgM).[2] In the case of immunoglobulin light chain MGUS, the involved immunoglobulin light chain (kappa or lambda) must be elevated and the monoclonal protein in the urine must be less than 500mg/24 hours.[2-4]

MGUS is common and found in 3.2% of the general population, with a prevalence that increases with age: 0.3% of the population younger than 50 years will have this protein, while up to 8% of the population above the age of 80 will have a detectable monoclonal protein.[5-6] There are considerable ethnic differences in the prevalence of the condition and it is more common and appears at a younger age in African Americans.[3,6-7] The main clinical significance of MGUS has been due to its risk of progression to multiple myeloma, amyloidosis or Waldenstrom's macroglobulinemia. It has been argued that MGUS is a necessary prerequisite for the development of multiple myeloma since retrospective analysis of serum samples from patients who subsequently developed multiple myeloma were found to have the signature monoclonal protein present sometimes for years in advance.[8-9] However, this observation is not unexpected since kinetic studies from the 1960s showed that for a clonal plasma cell to grow and reach a clinically significant size, more than a

decade has to pass.[10] This seminal observation has been confirmed with more recent analysis.[11] Various models have been developed to try and determine the risk of progression to multiple myeloma at the time of diagnosis and provide some guidance on the frequency of follow up in this condition. Part of the problem with this approach relates to rigid cut-offs that have been imposed on the diagnostic categories (MGUS, smoldering multiple myeloma and multiple myeloma). The result is that there are patients who do not meet these rigid criteria and in such situations (as always) the clinical judgment of the physician becomes essential rather than following guidelines blindly.

In clinical practice, the diagnosis of MGUS may appear to be easy – the presence of the monoclonal protein without the 'CRAB' features that define multiple myeloma (hyperCalcemia, Renal failure, Anemia and Bone lesions). Moreover, there is the impression that when the size of the monoclonal protein is small (especially when much smaller than 30g/L), then the risk of multiple myeloma is trivial or non-existent and the label of MGUS quickly applied. This can expose the patient to potential harm for several reasons: (i) Such thinking will inevitably exclude patients with light chain multiple myeloma (maybe up to 10% of patients), (ii) eliminate from consideration patients with oligosecretory or non-secretory multiple myeloma (~3%), (iii) exclude the possibility of AL amyloidosis, and (iv) likely exclude from consideration important clinical conditions associated with MGUS. The list of 'benign' conditions associated with MGUS keeps increasing.[12] Therefore MGUS should only be diagnosed if the physician consciously excludes these conditions with a comprehensive history and physical examination and targeted testing as clinically indicated. The purpose of this review is to highlight the better known associations between MGUS and other conditions and discuss the relevant clinical features of these syndromes so as to increase awareness of these associations and facilitate diagnosis and therapy. Some of these associations lead to well defined clinical syndromes affecting several organ systems while other manifestations are restricted to single organs. It is important to emphasize that in general there is no linear association between the size of the monoclonal protein and the presence of associated diseases or syndromes and therefore, it is the presence of the monoclonal protein that should be considered as an aid to the diagnosis or exclusion of these specific conditions.

Multisystem

AL or AH amyloidosis

Almost 90% of all patients with amyloidosis will have a syndrome related to the deposition of immunoglobulin light (AL) or heavy chains (AH) or both (AL/AH).[13] The deposited protein appears as an amorphous material on hematoxylin and eosin (H&E) staining and demonstrates apple-green birefringence with Congo red staining under plane polarized light. Diagnosis of amyloidosis depends on recognition of the various clinical syndromes that patients can present with. The major organs affected (alone or in combination) are the heart (restrictive cardiomyopathy), the kidneys (nephrotic syndrome), peripheral neuropathy, gastrointestinal involvement (upper gut: macroglossia, dysphagia, early satiety, unexplained nausea and vomiting; lower gut: constipation or diarrhea alternating with constipation), weight loss, carpal tunnel syndrome, liver involvement (non-tender hepatomegaly; elevated alkaline phosphatase), autonomic dysfunction, purpura (typically periorbital), and vascular disease (lower extremity or jaw claudication).[14-15] In patients with symptoms of heart failure without hypertension or diabetes mellitus, amyloid cardiomyopathy is an important consideration, especially if there is left ventricular hypertrophy or a pseudo-infarction pattern on the electrocardiogram.[15] The same approach applies to patients with unexplained nephrotic range proteinuria. The urine protein electrophoretic pattern can provide an important clue: if the protein is mainly albumin, AL amyloidosis needs to be excluded. If however, the protein is mainly due to the monoclonal immunoglobulin or light chains ('Bence Jones proteinuria') then multiple myeloma is much more likely. Although it is more common to have lambda light chains as a cause of amyloidosis, this cannot be used to exclude this disease. Indeed, perhaps 30% of patients with AL amyloidosis may have immunoglobulin kappa light chains. Diagnosis of amyloidosis depends on a tissue biopsy and it can never be excluded simply because the serum protein electrophoresis and immunofixation are negative. Nor is it excluded if the serum immunoglobulin free light chains are normal since patients can have non AL amyloidosis

or (rarely) even AL amyloidosis. The most accessible organs for biopsy include the bone marrow and subcutaneous fat – together they can identify amyloidosis in up to 90% of patients.[13] If these are negative, then a biopsy of the major affected organ (e.g. kidney or heart) will yield the diagnosis. It is critical to identify the amyloidogenic protein properly to ensure appropriate therapy. Specifically, the presence of a monoclonal protein in the serum or urine does *not* guarantee that the amyloidosis is due to the plasma cell disorder and typing is the only definitive way to determine the identity of the causative protein.[16] This is ideally done by laser capture micro-dissection followed by MALDI-TOFF mass spectrometry.[17] Therapy of AL amyloidosis is complex and depends on the organs affected, their severity as well as the performance status of the patient.[13,15,18] Although in many patients with AL amyloidosis the clonal plasma cell burden in the bone marrow is less than 10%, others may have higher fractions of plasma cells. Patients with a low plasma cell burden may be eligible for autologous stem cell transplant if they meet strict selection criteria.[18] Other patients can be treated with a bortezomib based regimen.[18] Ideally, such patients should be seen at a center of excellence for optimal staging and planning of care. It has to be emphasized that a patient with MGUS may have TTR amyloidosis affecting the heart and this possibility increases with older age. Therefore, if the amyloidosis syndrome appears to be restricted to the heart, a pyrophosphate scan should be performed to determine whether the amyloid deposits are TTR versus immunoglobulin related. If in doubt, then a cardiac biopsy should be performed to avoid the possibility of treating a patient with TTR amyloidosis using chemotherapy. This becomes also more relevant with the availability of tafamidis that can treat TTR amyloidosis.

POEMS syndrome

Patients who present with a progressive sensory or sensory-motor polyneuropathy should be tested for the presence of a monoclonal protein to diagnose or exclude the POEMS syndrome. This is an acronym standing for **P**olyneuropathy, **O**rganomegaly, **E**ndocrinopathy, **M**onoclonal protein and **S**kin changes.[19] The neuropathy and monoclonal proteins are cardinal features of the syndrome. Almost invariably, the M-protein is lambda light chain restricted and often is quite small in size.[20] Immunofixation of the serum is essential so as not to miss the small protein that is critical for diagnosis. The neuropathy is typical of a chronic inflammatory demyelinating type and can progress over a matter of weeks. Involvement of the respiratory muscles is common and many patients may require supplemental oxygen for dyspnea or hypoxemia. Pulmonary function studies show a 'restrictive pattern' with low inspiratory and expiratory pressures due to respiratory muscle weakness. Hepatomegaly, splenomegaly and lymphadenopathy are common. The endocrinopathies vary and can affect any endocrine system of the body with hypogonadism being the most common.[21] The skin changes are protean and include clubbing, leukonychia, hypertrichosis, hyper or hypopigmentation and cherry angiomas as well as acrocyanosis.[22] Other features are captured by a second acronym: PEST. **P**apilledema is common in the absence of intracranial pathology. **E**ythrocytosis and **T**hrombocytosis and also not infrequent and sometimes patients are diagnosed with a myeloproliferative neoplasm. However, careful examination of the bone marrow as well as molecular testing will exclude clonal erythrocytosis or thrombocytosis.[23] **S**erositis is common leading to fluid retention either as lower extremity edema or ascites. Pleural effusions are frequent even in the absence of heart disease. Vascular endothelial growth factor (VEGF) levels are often quite high in POEMS syndrome and in the correct clinical context are highly suggestive of the diagnosis although the test is not specific.[24] Proper therapy of patients with POEMS syndrome is associated with a rapid decline and normalization of VEGF. Patients typically have sclerotic bone disease (in contrast to the lytic lesions of multiple myeloma) that are best detected by PET/CT. Therapy of POEMS syndrome depends on whether the cause is an osteosclerotic plasmacytoma or diffuse infiltration of the bone marrow by clonal plasma cells (often in low numbers). For a solitary sclerotic lesion, radiation therapy is the standard of care but if there is diffuse marrow involvement, chemotherapy or autologous stem cell transplantation is the best approach.[25] The peri-transplant care of these patients can be quite challenging due to their multisystem disorder, problems with fluid retention and respiratory muscle weakness. These patients seem to be a significant risk of peri-engraftment syndrome that

needs to be promptly diagnosed and treated with glucocorticosteroids.

Idiopathic Capillary leak syndrome (Clarkson's disease)

Patients with Clarkson's disease present with repeated episodes of hypotension followed by fluid retention.[26] Often the patient is diagnosed with dehydration and given large volumes of intravenous fluids to raise the blood pressure. However, a careful history will fail to find any reasonable mechanism for the dehydration. The clue to the diagnosis is the presence of hemoconcentration (elevated hemoglobin and hematocrit) with concomitant low serum protein and albumin concentrations: this combination is pathognomonic of the phenomenon of capillary leak. The patient will have no evidence of chronic liver disease, nephrotic syndrome or a protein losing enteropathy that would otherwise explain the low serum protein and albumin. Nor would there be any evidence for an acute infectious or inflammatory insult that could lead to extravasation of fluid and protein. Over the course of a few days these laboratory anomalies will return to normal as the episode of capillary leak resolves. The mechanisms that trigger these sporadic and potentially life threatening episodes are unclear and they can occur anywhere and at any time. Patients with this syndrome will have a monoclonal protein that in the proper context would help establish the diagnosis. Often patients are misdiagnosed as septic shock or with angioedema, cardiac dysfunction or autonomic failure but a complete blood count and serum albumin and protein during an acute episode provide the evidence for hemoconcentration and in the presence of the monoclonal protein (typically an IgG kappa)[27] establish the diagnosis. Therapy is with monthly intravenous immune globulin that is generally very effective.[28] Other patients may be treated with beta agonists as well as theophylline.[29]

Scleromyxedema

Scleromyxedema is a multisystem disorder due to the deposition of immunoglobulins in various organs including the skin (waxy papules or plaques) and internal organs such as the gastrointestinal tract, the heart and rarely the central nervous system. Patients often present with joint and skin stiffness, tightening and thickening of the skin, dysphagia, or even seizures. When restricted to the skin, the condition has a better prognosis. Involvement of the heart, lungs or brain is associated with a guarded prognosis. It has similarities to systemic sclerosis except for the waxy papules that may occur on the fingers, behind the ears, or over extensor surfaces (e.g. elbow). Patients will also have a monoclonal protein in their serum but typically there will be very few plasma cells in the bone marrow.[30] The disease can be rapidly progressive and therefore prompt recognition is important. Immunomodulatory drugs such as lenalidomide are highly effective. Patients may also have an excellent and prolonged response to autologous stem cell transplantation.[31] Intravenous immune globulin may also be effective, although it has to be given long term.[32]

TEMPI syndrome

A syndrome characterized by **T**elangiectasis, erythrocytosis with elevated **E**rythropoietin levels, **M**onoclonal protein, **P**erinephric fluid collections and **I**ntrapulmonary shunting was initially reported in 2011 and since then several more cases have been described.[33-34] The patient may have extreme erythrocytosis and with a very high erythropoietin level suggesting a reactivbe increase in erythrocytes. Patients will have hypoxemia due to intrapulmonary shunting which may be quantitated using radionuclide approaches. The causative role of the plasma cell disorder in this syndrome is supported by resolution of symptoms and sign with therapy directed at the clonal plasma cells. Bortezomib based therapy[35] or even autologous stem cell transplantation[36] may lead to long term disease control.

Monoclonal gammopathy of renal significance

The nephrotoxic potential of monoclonal proteins have been known for a long time. The best characterized is myeloma cast nephropathy where monoclonal immunoglobulin light chains form casts that obstruct the renal tubules leading to renal failure.[37] This is a common presentation of multiple myeloma and perhaps 30% of patients will present with primarily renal dysfunction as the sole manifestation of multiple myeloma. These patients typically have very high monoclonal protein levels both in the urine and serum. Very high serum immunoglobulin free light chains in a patient with renal failure are highly suggestive of myeloma cast nephropathy and signal the need for urgent therapy

to salvage kidney function.[38] However, monoclonal proteins can cause a wide variety of renal pathologies, even when present in low concentrations. AL amyloidosis was discussed earlier in this review. More recently, the concept of monoclonal gammopathy of renal significance (MGRS) was proposed and it encompasses a spectrum of renal conditions.[39]

Membranoproliferative glomerulonephritis

In a recent series of patients with membranoproliferative glomerulonephritis (MPGN) who did not have any associated infection, connective tissue disorder, any evidence of complement dysregulation or other malignancies, 41% were found to have monoclonal deposits (usually IgG3) and complement that were identical to those in the circulation and/or urine and of the same isotype as the clonal plasma cells in the bone marrow.[40] These deposits appear granular on histology and present with the typical hematuria proteinuria, hypertension and impaired renal function. Often the monoclonal protein is kappa light chain restricted. Therapy directed at the plasma cells is critical to salvage kidney function. A bortezomib based regimen such as cyclophosphamide, bortezomib and dexamethasone (CyBorD) is generally recommended.[41]

Immunotactoid glomerulonephritis

Patients with this condition present with a 'nephritic syndrome' namely, an active urine sediment that includes hematuria and proteinuria, azotemia and hypertension. Biopsy of the kidney shows organized microtubular deposits of immunoglobulins, typically IgG in contrast to the disorganized monoclonal deposits seen in *monoclonal immunoglobulin deposition disease*.

Acquired Fanconi syndrome

This syndrome is normally suspected in patients with hypophosphatemia with a low potassium and low serum uric acid levels in the context of significant azotemia. A metabolic acidosis without an anion gap is also present. As an acquired type of renal tubular acidosis, patients with have glycosuria and aminoaciduria. Most often, patients have a kappa light chain restricted monoclonal protein and they may have immunoglobulin crystals in the proximal tubules on renal biopsy. Therapy of the underlying plasma cell disorder often leads to improvement of the acquired Fanconi phenotype.[42]

Neurologic syndromes

Sensorimotor peripheral neuropathy

In patients who present with an acquired, distal demyelinating and symmetric neuropathy, monoclonal protein should be performed, especially if a clear etiology of the neuropathy (e.g. hereditary, diabetes mellitus, alcohol and medication effect) cannot be established. The strongest association between MGUS and neuropathy is for patients with a monoclonal IgM. Many of these patients will have anti-myelin associated glycoprotein (MAG) antibodies, although these are not specific. Non-IgM related neuropathy often presents as length dependent sensory motor neuropathy to classic chronic inflammatory demyelinating polyneuropathy (CIDP) with predominantly motor nerve involvement. Often patients with IgM related neuropathy will have features of demyelination on EMG while IgG related neuropathy is often associated with electro-diagnostic features of axonopathy. In all these patients, AL amyloidosis and POEMS syndrome need to be excluded. Given the relatively high frequency of MGUS in the population above the age of 50, it has been difficult to prove a causal association between MGUS and neuropathy, except perhaps for IgM (43). Therapy is often unsatisfactory and includes intravenous immunoglobulin, plasmapheresis, rituximab or in patients with Waldenstrom's macroglobulinemia bendamustine with rituximab. Given the limited data of a link between IgG and CIDP, patients who present with the latter should be treated with standard therapy such as plasmapheresis, IVIG or glucocorticosteroids.

Sporadic late onset nemaline myopathy

This is a rare acquired myopathy that generally presents after the age of 40 years. Patients present with a sub-acutely progressive, proximal/axial (or less likely distal) muscle weakness often associated with dysphagia and respiratory muscle weakness. Symptoms may be asymmetric in some patients. Muscle enzymes are normal but the EMG shows fibrillation and myopathic features.[44] At least halve of these patients will have a monoclonal protein. Muscle biopsy establishes the diagnosis by the presence of nemaline structures. Various treatment options are

available including autologous stem cell transplant[45] or lenalidomide with dexamethasone that may lead to long term disease control.[46]

Skin

Acquired C1 esterase inhibitor deficiency

Activation of bradykinin release can lead to angioedema that is life threatening if it affects the tongue or upper airways. This results from the activation of the contact phase with kinin production in the absence of C1-esterase inhibitor. Although the most common cause of C1 esterase inhibitor deficiency is genetic, acquired forms of the disease exist and in one large series of 92 patients, a monoclonal protein was identified in 28.[47] Antibodies against C1 esterase inhibitor were found in 17 of these patients. Most of the patients had MGUS, although several had multiple myeloma or AL amyloidosis.

Cryoglobulinemia

Cryoglobulins are proteins that precipitate in a temperature dependent manner. Their thermal stability may be quite variable and as a result they may be asymptomatic or associated with severe symptoms. Classically, cryoglobulins are divided into 3 types: Type 1 with a monoclonal protein (IgG or IgM), Type 2 with a monoclonal protein (IgM) and polyclonal IgG with a positive rheumatoid factor assay and Type 3 with only polyclonal immunoglobulins. Type 1 is invariably associated with a clonal plasma cell disorder, typically multiple myeloma. Patients generally present with Raynaud's phenomenon although they can also have vasculitis symptoms such as skin ulcers, livedo reticularis, purpura mononeuropathy or mononeuritis multiplex as well as renal manifestations including azotemia, proteinuria or a 'nephritic syndrome'.[48] Patients with Type 1 or Type 2 cryoglobulinemia will have a detectable monoclonal protein. In general for these patients therapy directed at the plasma cell or lymphoproliferative clone is indicated.[48]

Schnitzler syndrome

Schnitzler syndrome (SchS) is a rare acquired inflammatory disorder characterized by recurrent episodes of 'urticaria' and the presence of a monoclonal protein, typically an IgM.[49-52] Other associated features include fever, an acute phase response (usually with florid elevation of the sedimentation rate and CRP), anemia, neutrophil leukocytosis as well as thrombocytosis. Many patients will have bone or joint pain and imaging studies, especially PET/CT may be helpful in defining the sclerotic bone lesions or the presence of the 'hot knee' sign that is often present.[53] Diagnosis is based on the 'Strasbourg criteria'.[50-51] Typically the urticaria will respond to systemic glucocorticosteroids but will recur once therapy is tapered. Often the skin lesions migrate from one location to another over the course of 48 to 72 hours and may be itchy. Biopsy of the skin lesions shows a predominantly neutrophilic dermal infiltrate without evidence of vasculitis. The serum ferritin is normal, in contrast to adult onset Still's disease. Complement levels are also normal and help to distinguish from hypocomplementemic urticarial vasculitis. Diagnosis of the syndrome is important since effective therapy in the form on anakinra, an interleukin 1 inhibitor, is highly effective. Patients will respond rapidly and completely to this therapy, although it will have to be given continuously.[50-52]

Necrobiotic xanthogranuloma

Patients with necrobiotic xanthogranuloma present with skin plaques that may be pink to red in color, or subcutaneous nodules with a predilection to occur around the orbit, flexural areas and the trunk.[54-55] In a significant number of patients, the lesions may ulcerate. Histologically, the dermis and subcutaneous tissue have a granulomatous infiltrate with bands of hyaline necrobiosis as well as Touton giant cells. Cholesterol clefts, lymphoid nodules with or without germinal centers, and focal accumulation of plasma cells may also be present. Helper T cells may be found in the granulomas. Immunologic studies will confirm that these are non-X histiocytosis. Many patients will have an associated monoclonal protein (usually Ig) and a significant number will have hyperlipidemia and low levels of C4. Therapy directed at the plasma cell disorder usually results in a complete resolution of the necrobiotic xanthogranulomas.[56]

Conclusion

The spectrum of conditions associated with monoclonal gammopathy is ever increasing[12] and recognition of the association between monoclonal proteins and other clinical features may assist in establishing the diagnosis of life threatening conditions. Although many patients with

monoclonal gammopathy may turn out to have MGUS and that simply need follow up, we cannot afford to miss important associated conditions. The size of the monoclonal protein has no predictive value with respect to the disease associations that need to be carefully excluded based on the old fashioned history and physical examination and targeted testing.

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Case Report

'Brenner tumour – The rare malignant variant'

Maria Petra Agius, Mandy Collict, Romina Cini Custo, Miljan Milic, Max Dingli, Albert Scerri

Abstract

Brenner tumors comprise an uncommon subtype of the surface epithelial-stromal tumor group of ovarian neoplasms. While most are benign, some can be malignant, and we report such a case and its treatment.

Maria Petra Agius MD*
Department of Obstetrics and Gynaecology
Mater Dei Hospital
Msida, Malta
mariapetra.agius@gmail.com

Mandy Collict MD Msc MRCSEd
Department of Obstetrics and Gynaecology
Mater Dei Hospital
Msida, Malta

Romina Cini Custo MD MRCOG DFSRH
Department of Obstetrics and Gynaecology
Mater Dei Hospital
Msida, Malta

Miljan Milic MD
Department of Pathology
Mater Dei Hospital
Msida, Malta

Max Dingli MD MRCOG
Department of Obstetrics and Gynaecology
Mater Dei Hospital
Msida, Malta

Albert Scerri MD FRCOG (UK)
Department of Obstetrics and Gynaecology
Mater Dei Hospital
Msida, Malta

**Corresponding Author*

Brenner tumours were first described by MacNoughton Jones in 1898 in his paper 'Uterine fibroid with anomalous ovarian tumour.'[1] In 1907, Fritz Brenner published his article 'Des oophorona folliculare' because of the resemblance of the epithelioid nests that Brenner found in his tumour, to Graafian follicles.[2] The semblance to Graafian follicles underpinned Fritz Brenner's classification of Brenner tumours as a variant of granulosa cell tumour. In 1932, Meyer, revisited the topic of Brenner tumours and clarified that they are different from granulosa cell tumours.[3] However, Brenner became aware of such a publication, 24 years later and did not publish any other articles on this subject.

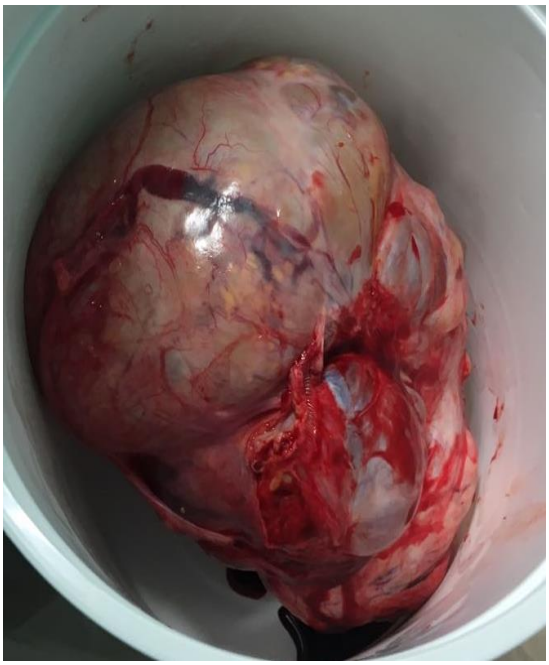
The incidence of ovarian cancer is 11.7 per 100,000 women per year.[4] Ovarian Brenner tumours represent a rare epithelial ovarian neoplasm accounting for approximately 1–2% of all ovarian tumours. Brenner tumours can be sub-classified into benign, borderline or malignant variants.[5] Malignant Brenner tumours are extremely rare, comprising less than 5% of all Brenner tumours. Malignant Brenner tumour was first reported in 1945 by von Numeras.[6]

A 70-year-old lady was referred by her family doctor with a large abdominal mass associated with a 19-kilo weight loss over 3 months, and 1-month history of constipation. General examination revealed a large hard mass, extending up to the umbilicus. An ultrasound scan showed a pelvic mass, probably of ovarian origin. Her Risk of Malignancy Index (RMI) score, which is calculated as the product of the ultrasound findings score, menopausal status and ca125 levels, added up to 1580. This was followed by computed tomographic assessment of the trunk, which revealed a large inhomogeneous mass measuring 16cm by 14cm by 17cm, originating from the pelvic cavity, with enlarged pelvic lymph nodes, together with an ill-defined hypovascular nodule measuring 8mm in diameter in the liver. The pelvic mass was further characterised by magnetic resonance imaging and was found to be arising from the left ovary.

Multiple peritoneal metastases and ascites were also seen. The liver was further studied by magnetic resonance imaging, which showed two indeterminate lesions in the right hepatic lobe. Ultrasound guided biopsy of the liver lesion was then performed. A core biopsy was obtained from the larger lesion and the histological and the immune-histochemical findings revealed metastatic carcinoma of urothelial nature. The immunohistochemistry profile was positive for BRCK, CK7, p63 and p53. It was inhibin and oestrogen receptor negative.

Total abdominal hysterectomy with bilateral salpingo-oophorectomy and omentectomy were performed via a midline laparotomy. Intra-operatively, an 18 cm-left ovarian tumour was found illustrated in Figure 1. On cross section, the mass showed a partly solid ossified tissue and partly cystic lesion. On sectioning of the cystic component, a multi-cystic lesion filled with thick yellowish fluid and multiple papillary structures were visible on the inner surface.

Figure 1: *Image showing the left smooth adnexal mass measuring 20cm by 13.5cm by 12.5cm. The fallopian tube is also visible, measuring 9cm by 3cm.*



The histological result confirmed a malignant Brenner tumour limited to the ovary, with angiolymphatic invasion (figure 2), pT1c3NxM1b, FIGO IVB**.**

Histologically, the left ovarian tumour was composed of sheets and nests of urothelial cells with intra-tumour micro cyst formation. Fused papillary fronds were focally identified projecting into the cyst lumina. Stromal desmoplasia was identified in areas, associated with focal dystrophic calcification and ossification. The urothelial cells showed mild-to-moderate nuclear pleomorphism and were briskly mitotically active as shown in figure 3. Areas of intra-tumoural necrosis were present.

The other ovary, uterus and cervix showed no evidence of involvement by tumour. The case was discussed at the Gynaecology Multidisciplinary Team Meeting and the patient was referred for further oncological care. The patient is currently receiving six cycles of adjuvant paclitaxel and cisplatin at 3-weekly intervals.

Figure 2: *Image illustrating vascular invasion of tumour cells.*

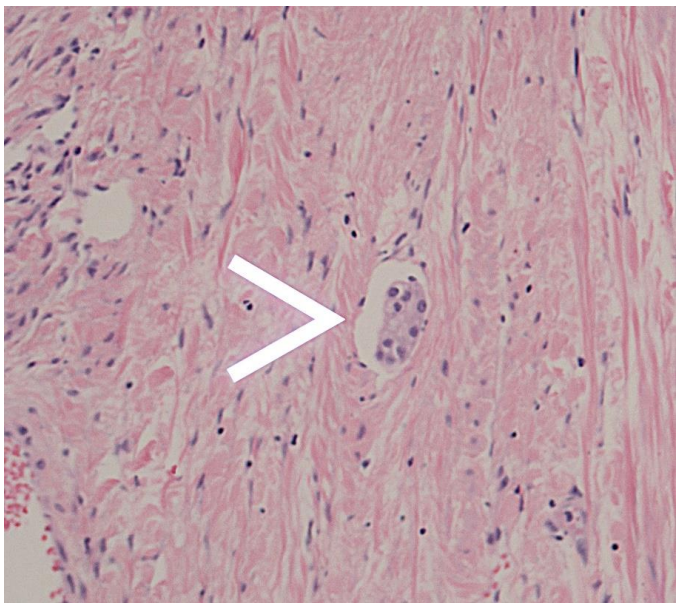
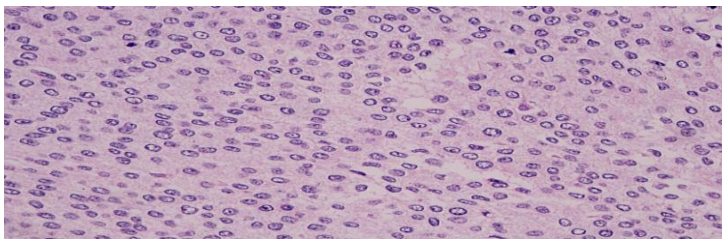


Figure 3: *Image illustrating actively dividing cell and prominent nuclei within cells.*



Hull and Campbell, in 1973, have proposed that a set of histological criteria should be present to diagnose malignant Brenner tumour. This requires the presence of malignant histological features forming irregular epithelial nests, benign or borderline Brenner tumour elements and stromal invasion.[7]

The prognosis of such malignancies is relatively poor in view of late presentation and rarity of the disease. In a number of case reports published by Yomomato et al. in 1999 [8], the tumour marker Ca72-4, was raised in both cases studied. Further studies need to clarify the validity of such a tumour marker. Ca125 can be used to monitor response to treatment and degree of disease burden, however, its use as a screening marker in Brenner tumours remains unclear.

In an analysis of 13 cases of malignant Brenner tumours, Gezginc et al. [9], found that the overall mortality was 50%, with a mean survival time post-diagnosis of 1 year. Surgery remains the mainstay of treatment in these cases. However, since malignant Brenner tumours are rare, management relies on published case reports and remains poorly defined.

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Case Report

Scurvy in children with autistic spectrum disorder: Not such a rarity

Jessica Coppini, Charles Borg, Cecil Vella

Abstract

Scurvy is an uncommon nutritional deficiency that results from low serum levels of vitamin C (ascorbic acid). Part of human history since ancient times, its incidence rapidly decreased following the discovery by Sir James Lind that citrus fruits can prevent it[1]. Despite this scurvy still exists today within certain predisposed groups.

We report three cases of scurvy that presented within a short time frame to the Paediatric Department in Mater Dei Hospital (MDH), Malta. All three children were known to have autistic spectrum disorder (ASD) with restricted diets.

A high index of suspicion together with appropriate history and examination can lead to timely diagnosis of this disease. In children deemed at-risk of developing scurvy we recommend routine screening for low serum levels of vitamin C.

Keywords

Vitamin C, Scurvy, Autistic Spectrum Disorder

Jessica Coppini MD.
jessica.coppini@gov.mt

Charles Borg MD, MRCPCH

Cecil Vella MD, MRCP(UK), FRCPC

**Corresponding Author*

Introduction

Scurvy is a nutritional deficiency of vitamin C that is rare in developed countries[2]. Its low incidence and non-specific presenting symptoms often leads to extensive investigations and delayed diagnosis.[1] At-risk groups of children include those with restricted diets stemming from psychiatric or developmental disorders, infants fed evaporated milk, haemodialysis patients and those receiving chemotherapy.[3-4]

Case Presentations

Case 1. A 4 year 11-month-old girl known to have Coeliac disease and autistic spectrum disorder (ASD) presented to the paediatric casualty with recent onset of a limp and left sided hip pain. The limp appeared one week after a viral upper respiratory tract infection; there was no associated trauma. A left-sided antalgic gait with no limb or joint deformity was observed on examination. Over the next few weeks the patient was closely followed up at the child outpatient clinic. On investigation inflammatory and infective arthritides were excluded and iron deficiency anaemia (IDA) was diagnosed. The initial working diagnosis was of a reactive arthritis.

Approximately one month after the initial presentation the patient re-presented to the paediatric casualty with easy bruising over lower limbs, an ulcer over her right big toe, diffuse hair loss, weight loss and a new ulcer over the left upper gum associated with gingivitis/contact bleeding. Further investigations included a bone marrow biopsy, multiple radiological imaging modalities and blood tests. An echocardiogram revealed a 3mm pericardial effusion. The patient continued to deteriorate clinically with a dropping haemoglobin, low grade fevers, persistent easy bruising, increased irritability and refusal to weight-bear having become bed bound.

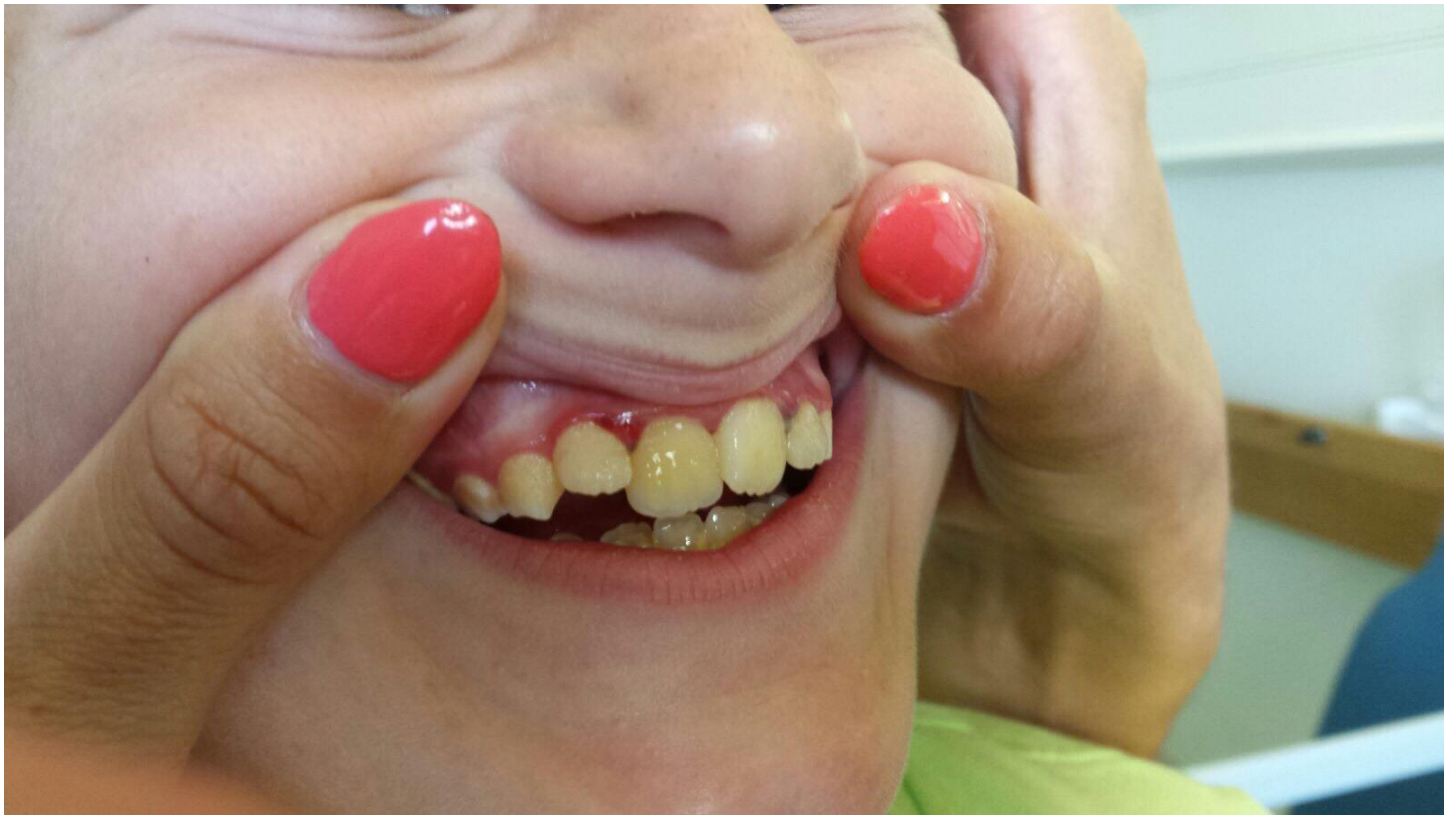
In view of the marked gingivitis, an ulcer of the lower lip, widespread petechial rash, bruising and joint pains, a vitamin C level was assessed

using High Performance Liquid Chromatography (HPLC) and found to be markedly low (<1.0mg/l, normal range 5.0-15.0mg/l). A diagnosis of scurvy was made. Her dietary history revealed years of an extremely restricted diet that had become further limited by her Coeliac disease. A dramatic and rapid improvement in all her symptoms was observed once high dose vitamin C supplementation at a dose of 250mg daily per oral was commenced.

Case 2. A 10-year-old girl, sibling to case 1, also autistic on a restricted diet was asymptomatic. However, routine screening showed a vitamin C (HPLC) level of <1.0mg/l (normal range 5.0-15.0mg/l) confirming the diagnosis of scurvy. She was started off on 20mg vitamin C supplements three times daily by mouth.

Case 3. A 9-year-old boy with ASD presented with an intermittent limp for two weeks. He was able to weight-bear, had no recent illnesses or trauma and on examination had no deformities. His parents described a one-month history of bleeding gums and a recent history of increased lethargy and decreased appetite on a background of a restricted diet. On examination gum hypertrophy and gingivitis (Figure 1) was found. Serum vitamin C (HPLC) levels were found to be <1.0mg/l (normal range 5.0-15.0mg/l). Within weeks of initiating oral replacement therapy, initially with 20mg vitamin C three times daily, his gum hypertrophy and bleeding resolved and his physical activity returned to normal.

Figure 1: *Clinical photograph of Case 3 showing inflamed and hypertrophic gums*



Discussion

Restricted interests and repetitive behaviour are core features of autistic spectrum disorder (ASD) and can result in feeding difficulties, restricted diets and a predilection for nutritional deficiencies including scurvy.[5]

Vitamin C found in citrus fruits and some vegetables (tomato, potato, cabbage, broccoli, lettuce, red peppers)[2] is an essential nutrient which humans are unable to synthesize *de novo*[1] with no stored form. Consequently, even just 1-3 months of an insufficient intake can lead to clinical manifestations of scurvy[4]. Vitamin C plays a major role in the synthesis and cross-linking of intercellular connective tissues, collagen, dentine and osteoid.[2] The resultant increased fragility of these tissues in scurvy explains many of its clinical manifestations.[6] Vitamin C plays other roles in human biochemistry as a cofactor, reducing agent and antioxidant[2] and enhances iron absorption.[1]

The earliest manifestations of scurvy are non-specific constitutional symptoms including lethargy, weight loss, decreased appetite and low-grade fevers.[2] Dermatological manifestations such as petechiae, ecchymoses, perifollicular haemorrhages and corkscrew hairs appear early on in the disease process. Gingival disease usually occurs next with swelling, bleeding, hypertrophy and loosening of teeth or poorly formed teeth.[7] In contrast to adults with the disease, musculoskeletal abnormalities are found in 80% of paediatric patients with scurvy[8] often resulting in an orthopaedic presentation. Joint pain/swelling, myalgias, haemarthrosis, muscular haematomas and fractures can occur resulting in limb pains, limping, leg weakness and/or refusal to weight-bear[6]. Iron deficiency anemia commonly coincides with scurvy secondary to decreased efficacy of iron absorption as well as iron losses from easy bleeding and bruising.[1] Cardiac hypertrophy, pulmonary hypertension and right heart failure have been reported in paediatric groups with scurvy[9] and could explain the persistent small pericardial effusion in Case 1. More advanced scurvy may present with bone marrow and adrenal suppression, psychological changes, poor wound healing and even death.[2]

The diagnosis of scurvy is often elusive, and a high index of suspicion combined with thorough history and examination is pivotal to confirming an early diagnosis without excessive and extensive investigation. Serum vitamin C levels (<2mg/L) are the gold standard for diagnosis, although radiological investigations provide complimentary evidence of the disease.[4] No radiological abnormalities were reported in our cases.

Ultimately, definitive confirmation of scurvy is the rapid resolution of symptoms once treatment with vitamin C supplementation is commenced,[7] with improvement of symptoms starting within 24 hours.[8] No specific treatment regime has been proposed for paediatric scurvy and the oral route is effective even in severe states of deficiency.[4] Weinstein et al. recommend oral doses of 100 to 300mg of vitamin C for paedatric scurvy patients until adequate serum levels are achieved -typically one month after commencing treatment.[8]

Conclusion

Scurvy is a rare disease associated with significant morbidity from the disease process itself and from the excessive sometimes invasive investigations that are undertaken if it is not considered in the differential diagnosis. Heightened awareness of this easily curable disease is especially important in patients suffering from ASD and others with restricted diets. We recommend screening for scurvy in all autistic patients known to have a restricted diet.

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An awkward looking pilomatrixoma

Juanita Parnis, Graham J Offer, Ngi Wieh Yii

Abstract

Pilomatrixoma is a rare and benign skin tumor of the hair follicle that tends to develop in the head and neck area. We report a case and briefly review the literature.

Case Report

This 30 year old gentleman who was treated for suspected pulmonary tuberculosis four years previously, presented to the plastic surgery clinic with a 3cm x 2cm, erythematous, fluid filled lesion on the left neck which had been growing for five months. On examination, the skin lesion had an underlying palpable 3cm mass. An ultrasound scan showed this to be a cystic lesion. Infectious diseases were consulted in view of the fact that this might have been cutaneous tuberculosis. However, fluid aspirates showed no signs of tuberculosis and an incision biopsy was indicative of a pilomatrixoma. The histology report from the complete excision came back as: "The specimen contains a benign pilomatrixoma".

A pilomatrixoma is also known as a pilomatricoma or a benign calcifying epithelioma of Malherbe. It is an uncommon slow growing benign adnexal skin tumour with a differentiation towards hair cells.

Juanita Parnis MD MRCS*
Plastic surgery Registrar,
Balmoral Level 3, Leicester Royal Infirmary, UK
Mater Dei Hospital,
Msida, Malta
juanitaparnis@gmail.com

Graham J Offer FRCS (Plast)
Consultant plastic and reconstructive surgeon,
Leicester Royal Infirmary,
UK

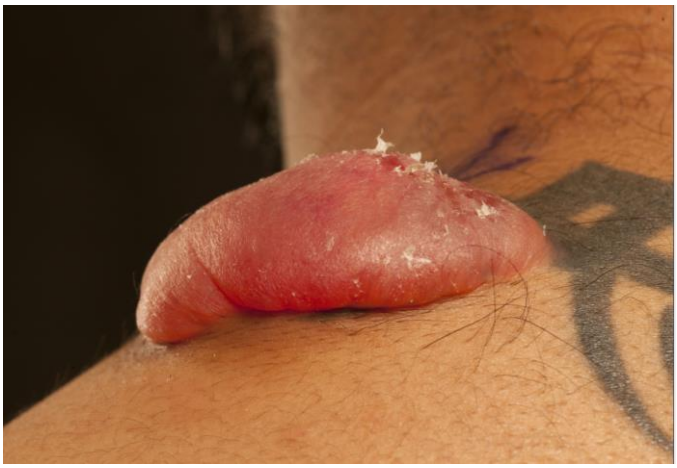
NgiWieh Yii FRCS (Plast)
Consultant plastic and reconstructive surgeon and Head of Department,
Leicester Royal Infirmary,
UK

**Corresponding Author*

Reports on the bullous variant of pilomatrixoma are rare. It occurs mostly on the shoulder and upper arms of females. It can also be found on the neck, trunk, eyelid, and scalp. The commonest age of presentation is between 10-20 years, and they vary between 1 and 3 cm in size. It usually presents as a flaccid red bulla with an underlying palpable hard mass.

Different theories have been proposed for the mechanism of bulla formation in pilomatrixoma. It could be due to mechanical irritation, a pseudo blister or production of elastinolytic enzymes which disrupt the collagen fibres and destruct and dilate the lymphatic vessels, which lead to accumulation of lymph fluid in the dermis causing a bulla.[1] Thus, bullous pilomatrixoma is also named as lymphangiectatic pilomatrixoma.[2]

Figure 1: *Bullous pilomatrixoma on the left shoulder*



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