MINIMA MEDICA

2023 ISSUE

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FOREWORD MESSAGE

SCOME Officer, *Ms Rebekah St. John*

I am extremely pleased to launch MMSA's Minima Medica Journal, which is back for a new edition in 2023! Ever since its inaugural edition, Minima Medica has been as a way for medical students to publish their research in a peer-reviewed journal. It also serves as a stepping stone for those who wish to enter the vast world of research but don't know where to start.

The 2023 edition of this journal includes 13 articles covering a large variety of medical topics, with each article being supervised and reviewed by two separate academics from the University of Malta, helping to strengthen the reliability and validity of Minima Medica as a reputable research journal.

This journal would not have been possible without the outstanding work of the team around it. I am extremely thankful for Andrea Cuschieri, the SCOME Publications Coordinator, who has been coordinating the entire process leading up to the words you will be reading shortly, from releasing and vetting article applications, to the coordination of the SCOME Research Conference at which this journal is being released. I would also like to thank Martha Schembri, the SCOME Assistant, whose unwavering commitment to the standing committee has shone through during every part of the year, and especially throughout this process. The MMSA PRO, Katrina Buhagiar, as well as PR coordinator Ryan Vella, should also be celebrated, as without their unique designing capabilities, this journal would not have existed. Finally, I would like to thank the many authors for putting the time and effort into writing and submitting the articles you are about

to read, as well as the academics who found the time to review each and every article. It is because of you that this journal is what it is.

I hope that every single reader, be they a student, academic, or otherwise, finds this journal to be a fruitful and interesting read, and that they enjoy delving deeper into some of the many marvels that the field of medicine has to offer.

FOREWORD MESSAGE

Editor-in-chief, Mr Andrea Cuschieri

MMSA's annual student journal, Minima Medica serves as a platform for medical students to share their scientific endeavours with their peers. Minima medica accepts all submissions, to ensure that all students have the opportunity to published their work without any hindrances. Yet, minima medica employed a blinded peer-review process, thanks to the contribution of local experts who graciously accepted our invitation to review this year's submissions. Thus, on behalf of minima medica I would like to extend my sincere appreciation, in no particular order, to the following academics:

- Professor Christian Scerri
- Professor Jean Calleja Agius
- Professor Pierre Schembri Wismayer
- Mr Ivan Esposito
- Professor Giuseppe Di Giovanni
- Professor Gary Hunter
- Professor Neville Vassallo
- Dr Daniel Vella Fondacaro
- Dr Alexander Borg
- Dr Michelle Ceci
- Dr Christian Zammit
- Mr Joseph Debono

I hope this year's instalment will inspire other medical students to conduct and publish their own research, while encouraging them to further pursue opportunities for their personal growth.

CASE STUDIES

Popliteal Artery Entrapment Syndrome

Author: *Thea Portelli*
Supervisor: *Professor Kevin Cassar M.D.,F.R.C.S.(Edin.),M.Med.(Dund.),M.D. (Aberd.),F.R.C.S.,F.F.St.Ed.*

Abstract

Ms X, a 22-year-old lady, was referred to the vascular unit in view of a long-standing history of bilateral exertional calf pain. Her pain-free walking distance was roughly 100 metres. Stopping to rest alleviated the pain within 5 minutes, allowing her to walk another 100 metres pain-free. The patient reported an insidious onset of these symptoms in 2016, with a gradual increase in time of onset from exertion over the next 2 years, to the current state she presented with, having remained stable since. On examination, the pedal pulses were palpable and on duplex ultrasound scanning (DUS), she had normal triphasic waveforms bilaterally in the distal arteries. There was no significant change in the waveforms on plantarflexion. However, a dynamic MRI-angiography (MRA) of the legs confirmed the diagnosis of a rare condition called popliteal artery entrapment syndrome (PAES). DUS was repeated with the addition of forced plantarflexion of the foot and on the right leg, triphasic waveforms changed to monophasic continuous with this manoeuvre. In view of this, Ms X underwent surgical exploration of the right popliteal fossa to release the popliteal artery from its entrapment.

Keywords: Popliteal artery; Popliteal artery entrapment syndrome

Fact file on PAES

PAES is a rare vascular disorder that can cause intermittent claudication (IC) in athletic young people without cardiovascular risk factors (1). Since it is rare and the symptoms it produces overlap with other, commoner disorders, diagnosing PAES can be challenging and it is not uncommon for patients to present with a long-standing history coupled with multiple unfruitful consultations (2). The overall incidence of PAES is believed to range from 0.17% to 3.50% within the general population (2), but this may not reflect the true prevalence due to mis- and undiagnosed cases (1). Active young males are predominantly affected (85%) and up to 30% of patients present with bilateral PAES (3).

Vascular compromise, often due to an underlying congenital anomaly within the popliteal fossa, results in an insidious and progressive onset of exercise intolerance. The patient is usually an athlete or active person in their 2nd-3rd decade of life (3), however, cases of patients as young as 7 years old have been reported (4). Patients often complain of reproducible calf pain that resolves quickly on cessation of exercise. The pain is typically in the back of the calf but if the anterior tibial artery is involved, it can also occur laterally or in the anterior leg (5). The pain and claudication distance vary for each patient and can range from mild enough that 'only' quitting their sport allows them to lead an otherwise

normal life (6), to severely disabling with a claudication distance of just a few metres. Apart from exertional pain, patients may also complain of pallor, coolness, paraesthesia, extremity numbness, foot pain, lower limb swelling and a muscular ache that persists for hours after cessation of exercise (7). Examination of a patient with PAES may reveal tight, hypertrophied calf muscles and absent foot pulses when the patient has been asked to walk a distance that normally reproduces their symptoms (8).

There are 6 subtypes of PAES, as demonstrated in Table 1. Types I–V are due to anatomical (congenital) causes and type VI describes acquired or functional cases in which the anatomy is normal. Symptomatic onset in both functional and anatomical PAES is thought to be related to a change in use of the gastrocnemius muscle, for example the commencement of athletic training, which results in muscular hypertrophy and overcrowding of the popliteal fossa. In functional PAES (fPAES), the muscular hypertrophy causes symptomatic compression of the popliteal artery with impaired blood flow during exertion. This results in repeated microtrauma of the muscles and subsequent development of constricting scar or fibrous tissue around the artery (9). Similarly, in anatomical PAES, muscular hypertrophy coupled with a pre-existing anatomical aberration, results in clinically significant and symptomatic impingement of the artery (10).

Diagnosis of PAES poses a challenge as there is no clear-cut consensus regarding the diagnostic workup (11). Ankle brachial pressure indexes (calculated by dividing the systolic blood pressure at the ankle by the systolic blood pressure at the arm) are often found to be less than the normal value of 0.9 in patients with anatomical PAES, but a false negative will likely be obtained if a patient has fPAES.

Table 1: An overview of the 6 popliteal artery entrapment syndrome subtypes (1).

Type I	An aberrant medial course of the popliteal artery around the normally-positioned medial head of gastrocnemius (MHG)
Type II	MHG attaches abnormally and more laterally on the femur causing the popliteal artery to pass medially and inferiorly
Type III	Abnormal fibrous band or accessory muscle arising from the medial or lateral condyle encircling the popliteal artery
Type IV	Popliteal artery lying in its primitive deep or axial position within the fossa, becoming compromised by the popliteus muscle or fibrous bands
Type V	The entrapment of both the popliteal artery and vein due to any of the causes mentioned above
Type VI or F	Muscular hypertrophy, resulting in a functional compression of both the popliteal artery and vein

Similar problems are encountered when using DUS on patients with fPAES, since at rest the artery is patent with normal waveforms. Occlusion, and therefore a significant change in waveform, only occurs during exertion and resolves soon after. Provocative protocols with active plantarflexion reduce the rate of false negatives but since not all patients with fPAES occlude in this position, there is still a risk of false negatives (12).

Magnetic resonance angiography (MRA) is a valuable modality in the diagnosis of PAES and should be considered after positive ultrasound studies to confirm the type of lesion, or negative studies with a remaining index of suspicion (11).

Untreated, PAES is potentially limb-threatening as it can lead to popliteal artery stenosis, thrombosis, distal arterial thromboembolism and in extreme cases, limb amputation (1, 13). After diagnosis, surgical correction of the anatomical aberration in PAES types I–V is therefore

always recommended. This involves surgical exploration of the popliteal fossa through a medial or posterior approach with myotomy of accessory slips, excision of occlusive fibrous bands or detachment of muscle. Within a few months from surgery, over 90% of patients report complete resolution of symptoms and are able to resume their sports or training (14).

Surgical treatment of fPAES remains more controversial since there is no clear anatomical entrapment that can be fixed but should be considered in patients with significant symptoms. Surgical outcome of fPAES is not as favourable as that of anatomical PAES, with only 77% of patients reporting resolution of symptoms after surgery (15). Guided botulinum toxin (Botox) injections appear to help some patients by causing localised muscle atrophy of the compressing gastrocnemius muscle.

Case report

Presenting complaint

A 22-year-old female patient complains of a 6-year history of bilateral calf pain brought on by walking, that is worse on the right, and is relieved by rest.

History of presenting complaint

The nature of the pain is a burning, fatigued sensation in the muscles that starts off on the posterolateral aspect of each leg and radiates to encompass the calf shortly after. The distance walked before onset of symptoms is remarkably constant at about 100 metres on the right and 150 metres on the left. This distance is reduced if the patient walks quicker than normal or at an incline. Stopping to rest alleviates the pain within 5 minutes, allowing her to walk the same distances before renewed onset of symptoms. If the patient does not stop to rest but keeps on walking through the pain, she experiences paraesthesia in her right toes and limping.

Ms X dates the onset of these symptoms to roughly 6 years earlier. Her earliest recollection of this pain is a cramping sensation in her calves at the end of 90-minute-long football training sessions. Over the course of 2 years, the walking distance regressed, and pain levels increased, forcing her to give up sports. The symptoms have remained constant since 2018.

Previous medical/surgical history

Ms X suffers from Hashimoto's thyroiditis since she was 8 years old. When she was 15, she was diagnosed with polycystic ovarian syndrome. At 18, Ms X was admitted to the psychiatric ward for suicidal plans after suffering for years from undiagnosed depression.

Drug history

The patient has no known drug allergies. Her drug history is listed in table 2.

Family history

Her father suffers from type 2 diabetes mellitus and hypertension. There is a history of Hashimoto’s thyroiditis and coeliac disease in both first- and second-degree relatives.

Social history

The patient is a non-smoker and does not drink alcohol or use illicit drugs. She lives at home with her family and is currently a university student. The symptoms interfere with daily life and she gives the following examples: going to the local pharmacy to buy her medications or crossing the university campus from one lecture to another cause pain.

Table 2: Ms X's drug history.

Drug	Dose	Frequency	Formulation	Reason for prescription
Thyroxine	100 micrograms	Once daily	Oral tablet	Thyroid replacement therapy
Escitalopram	20 mg	Once daily	Oral tablet	Major depressive disorder
Trazodone	100 mg	Once daily	Oral tablet	Major depressive disorder
Metformin	500 mg	3 times daily	Oral tablet	Polycystic ovarian syndrome

Systemic enquiry

Nil to note.

Differential diagnosis

1. Intermittent claudication:
    - This is typically secondary to peripheral arterial disease but due to the patient's age and lack of cardiovascular risk factors, unlikely to be the aetiology of the symptoms.
    - Rarer causes of IC include PAES and cystic adventitial disease of the popliteal artery. However, IC typically resolves within 1-2 minutes of ceasing exercise. In this patient's experience, it takes less than 5 minutes but more than 1-2 minutes.
2. Chronic exertional compartment syndrome (CECS):
    - This occurs in susceptible individuals when during exertion, an increase in muscle volume within a rigid fascial compartment causes a marked raise in intra-compartmental pressure. This results in exertional muscular pain (16). CECS most commonly affects the anterior compartment of the lower limb (17).
    - Symptoms are similar to those of PAES, with two exceptions. Firstly, peroneal nerve dysfunction may occur in CECS, which is not typical of PAES. Secondly, after exertion brings on the pain, longer periods of rest (sometimes 30 minutes) are required to alleviate it in CECS. However, recent case reports show that a significant number of patients with CECS also have PAES, and so in practice these differences may not be so relevant (18, 19).
3. Neurogenic claudication:
    - This is caused by lumbar spinal stenosis. Symptoms include pain, weakness and paraesthesia that extend into one or both legs and/or lower back. These occur with walking, standing or extension of the back.
    - However, patients with neurogenic claudication typically report an improvement in symptoms when walking uphill or flexing their spine (20), neither of which apply to this patient.

Physical examination and preliminary investigations

On examination, the lower limbs had no stigmata of peripheral vascular disease. They were symmetrically warm with normal capillary refill time. The pulses were palpable throughout both lower limbs.

Preliminary investigations included DUS. This ruled out cystic changes in the popliteal artery and revealed triphasic waveforms, both at rest and with plantarflexion, in the distal arteries and popliteal artery of both limbs.

Diagnostic investigations

1. MRI of the whole spine. This test was ordered to exclude potential underlying pathologies such as lumbar spinal stenosis and spinal disc herniation.
2. MRA of the legs with and without manoeuvres. This test involves two sets of imaging; one at rest and another after the patient performs provocative movements until the symptoms are produced. MRA was chosen as a first-line investigation since it is non-invasive, unlike angiography.

Findings and Diagnosis

1. The MRI of the whole spine showed no pathologies or abnormalities.
2. The MRA images obtained after performing provocative manoeuvres showed evidence of PAES at the level of the knee in both legs (Figure 1). As no apparent anatomical cause was observed for this entrapment, a diagnosis of fPAES was made.
3. Following the MRA, DUS was repeated with the addition of forced plantarflexion of the foot. On the left, no changes were apparent; however, on the right, waveforms changed dramatically from triphasic to

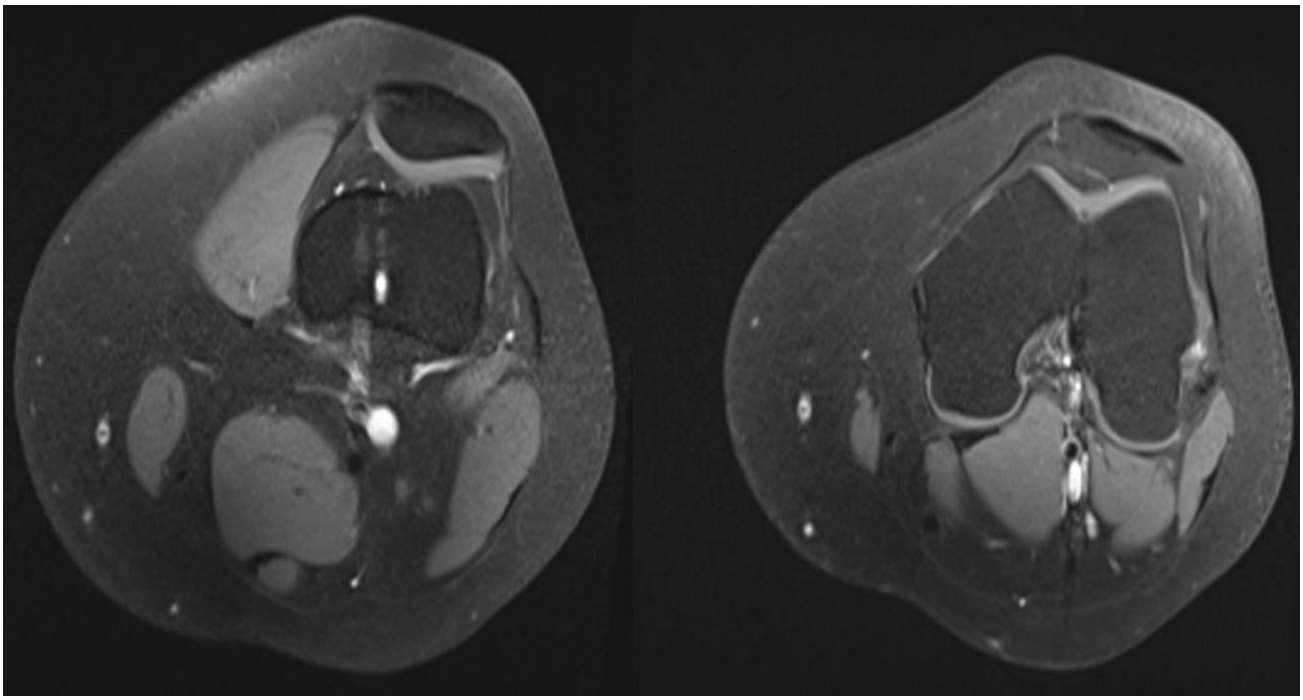

Figure 1: Consecutive sectional images of the patient's left knee. The image on the left shows the popliteal artery (white) prior to entrapment; note its round shape. On the right, the popliteal artery is entrapped between the medial and lateral heads of the gastrocnemius muscle at the level of the femoral condyles; note its elliptical shape. Further down the leg, the artery resumes its round cross-sectional shape.

monophasic continuous with this manoeuvre.

4. Concomitant CECS complicating the diagnosis of fPAES could not be ruled out due to a lack of reliable diagnostic modality.

Management

After discussing the diagnosis with the patient, it was decided to manage fPAES alone initially, with the possibility of revisiting CECS as a potential diagnosis and treatment option in the future in case the symptoms persisted.

Surgical exploration of the right popliteal fossa was carried out with the patient under general anaesthesia. Intraoperatively, the entrapment seen on MRA was confirmed again by the bottleneck appearance of the popliteal artery. The artery was successfully dissected free from a fibrous web constricting it into this shape and myotomy of the MHG was performed to create more space in the fossa.

The patient was encouraged to mobilise a few hours after surgery and happily reported no symptoms on the right when doing so. She was discharged the following day to continue recovery at home.

Follow-up

Unfortunately, during a post-operative follow-up, Ms X reported that her symptoms had begun to return approximately three weeks after surgery. Despite this development, DUS revealed triphasic waveforms in the distal arteries during forced plantarflexion; a significant improvement compared to the pre-surgery monophasic waveforms. It was thus deemed likely that the patient was also suffering from CECS, and a referral to the orthopaedic team for further management was made.

Declarations

Conflict of interest: N.A.
Ethical statement: Consent for publication from Ms X was obtained.

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Case report on Caesarean Scar Ectopic Pregnancy

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Supervisors: *Dr. Judith Marie MifsudMD, EFOG-EBCOG, MSc.; Dr Max Dingli MD MRCOG*

Abstract

A 42-year-old woman presented with symptoms of vaginal bleeding and lower abdominal pain. The patient was diagnosed with a Caesarean Scar Ectopic (CSP) pregnancy, which is a type of ectopic pregnancy (EP) that occurs in women who have undergone previous Caesarean sections. EP is the leading cause of maternal morbidity and/or mortality during the first trimester, with a prevalence of 11 per 1000 pregnancies in the UK. The incidence of CSP has increased globally as the incidence of Caesarean sections has risen. Diagnosis of EP is undertaken by carrying out imaging tests, which confirm the presence of a gestational sac outside the endometrial cavity. The text also discusses the risk factors associated with the occurrence of EP, including age above 35 years, history of endometriosis, pelvic inflammatory disease, cigarette smoking, use of progesterone-only contraception, previous EP, and previous pelvic surgery. The patient was administered systemic intramuscular methotrexate, but the hCG levels continued to rise, and she subsequently underwent administration of gestational sac methotrexate under transvaginal ultrasound guidance. The hCG levels down trended following the procedure, and the patient was discharged home within 48 hours, with no complications reported.

Keywords: case report; ectopic pregnancy; methotrexate

Case History

A 42-year-old lady, para 2+0, presented with a 2-day history of minimal vaginal bleeding and minimal lower abdominal pain. She had no shoulder-tip pain. She complained of slight nausea but no vomiting. The patient was amenorrhoeic for 5 weeks 2 days, and a urinary pregnancy test done 1 week before was positive. The abdomen was soft, with discomfort suprapubically, and no peritonism. No masses were palpable. She had undergone an emergency lower segment Caesarean section LSCS 6 years previously, at 30 weeks gestation, for severe intrauterine growth restriction. The surgery was complicated by cardiac arrest and atrial fibrillation, with spontaneous cardioversion. She had undergone a second elective (LSCS) 5 years previously at term with no complications. She also underwent a total thyroidectomy 1 year previously for Graves hyperthyroidism and a papillary microcarcinoma. Following

the surgery, she had multiple admissions in view of Hypocalcaemia. Currently she was taking the following medications: Thyroxine 25mcg daily; Calcium 500mg TDS; Vitamin D 20,000 IU weekly.

Transvaginal ultrasound of the pelvis showed an anteverted, retroflexed uterus with an endometrial lining of 7.5mm. There was no intrauterine gestational sac. Just below the scar and to the left of the midline, a 14mm vascular hyperechoic ring with a hypoechoic centre was seen, containing a yolk sac (Figure 1). There was no free fluid in the pouch of Douglas. The right ovary appeared normal and there was a corpus luteum on the left ovary, as shown in the original ultrasound below.

She subsequently underwent a pelvic MRI which confirmed the absence of an intrauterine gestational sac and an abnormal soft tissue mass measuring 1.5x1.3cm within the previous uterine LSCS scar. The shortest distance to the uterine serosa was 3mm and a scar ectopic was confirmed.

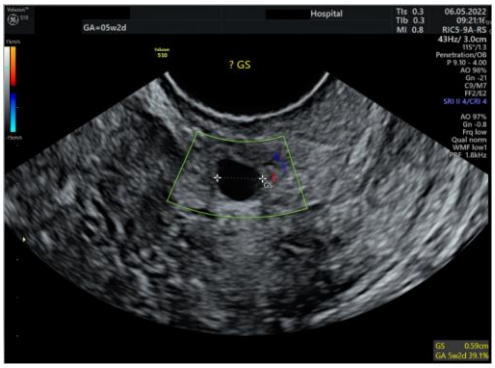

Figure 1: Transvaginal Ultrasound of the pelvis

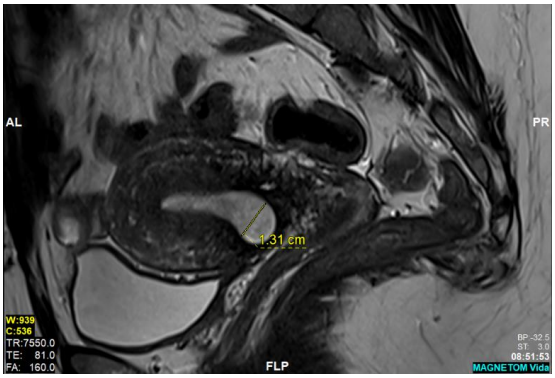

Figure 2: Pelvic MRI

HCG levels showed a suboptimal rise over 5 days from 146 to 331mIU/mL and there was mild anaemia. Thus, a Caesarean scar ectopic was confirmed.

Management

She was administered systemic intramuscular methotrexate after the diagnosis, however the hCG levels continued to rise to 933mIU/mL. After 8 days and following appropriate consent, the patient underwent administration of gestational sac methotrexate under transvaginal ultrasound guidance. A dose of Ativan was administered 1 hour prior to the procedure. A single dose of 50mg/m^2 Methotrexate was injected under sterile conditions. A needle guide was attached to the ultrasound probe and a double-lumen ovum aspiration needle was used. The patient complained of minimal discomfort during the procedure and was discharged home within 48 hours, following administration of simple analgesia. hCG levels downtrended following the procedure and returned to normal within 6 weeks. There were no complications reported following the procedure.

Discussion

Ectopic pregnancy (EP) occurs when a fertilized ovum implants itself outside of the endometrial cavity. This is the leading cause of maternal morbidity and/ or mortality during the first trimester. Diagnosis of EP is to be done early on during pregnancy, to avoid the development of life-threatening complications. The incidence of EP is 11 per 1000 pregnancies in the UK (1). About 1.5-2.1% of patients undergoing IVF are affected by ectopic pregnancies (2).

EP may be tubal or non-tubal. Tubal EP involve the blastula being implanted within the fallopian tube including the isthmal, ampullar or infundibular region. Non-tubal EP include cervical, cornual, ovarian, Caesarean scar and abdominal pregnancies, the latter being the rarest presentation (3).

A number of risk factors are associated with the occurrence of EP, including: age above 35 years; history of endometriosis, pelvic inflammatory disease (PID)- most commonly *Chlamydia trachomatis* and *Neisseria gonorrhoeae*; cigarette smoking, the use of the progesterone only contraception, previous EP and previous pelvic surgery (4)(5).

Caesarean Scar Ectopic Pregnancy

Caesarean Scar Ectopic Pregnancy (CSP) is the occurrence of a gestation surrounded by the myometrium and fibrous tissue of a previous caesarean section scar (6). It is documented that CSP occurs in 1:2226 of all pregnancies, and account for 6% of all ectopic pregnancies in women who have undergone previous Caesarean sections (7). The prevalence of Caesarean Section has globally increased from 12% in 2000 to 21% in 2015. This increase in rate of CS reflects the increase in incidence of CSP (8).

Diagnosis of CSP

Diagnosis of EP is undertaken by carrying out imaging tests, which confirm the presence of a gestational sac outside the endometrial cavity. Transvaginal ultrasonography is the first-line tool; the Royal College of Obstetricians and Gynaecologists (RCOG) suggest the following criteria are met in order to diagnose a CSP, the following criteria need to be fulfilled: empty uterine cavity (9); the gestational sac or trophoblast is embedded at the site of the caesarean scar, located anteriorly at the level of the internal os (10); between gestational sac and bladder, there either is a thin layer of myometrium or no myometrium at all (9) (11); Doppler exam confirms the presence of a trophoblastic or placental circulation (12) and empty endocervical canal (9).

Magnetic Resonance Imaging (MRI) is a second-line tool and helps to confirm the diagnosis; however, it is more expensive and is less readily available when compared to ultrasonography. Local expertise to interpret the MRI findings are also necessary to make the correct diagnosis. The biochemical investigation of serum beta- human chorionic gonadotrophin (hCG) level measurements is a useful investigation to make a diagnosis of pregnancy and to monitor treatment outcomes.

Management of CSP

Management of CSP involves conservative, pharmacological, and/or surgical treatment.

Pharmacological treatment involves administration of methotrexate. Methotrexate is a folic acid antagonist, which aids to reduce the inflammation present during an ectopic pregnancy by two biochemical pathways. This first pathway is via Adenosine release, due to its anti-inflammatory properties (13). The second pathway is the Inhibition of DNA synthesis which acts as a trophoblast growth inhibitor (14). This occurs via inhibition of transmethylation pathways which indirectly inhibit the S-phase of cell division. This process occurs by halting the formation of tetrahydrofolate, the coenzyme used in several transmethylation reactions (10).

Administration of methotrexate in cases of CSP can be done via the intramuscular route, or via the local route by injecting the drug directly within the gestational sac under ultrasound guidance. Intragestational sac administration of methotrexate is a more effective way to terminate the pregnancy, since it is more fast acting and has less side effects than intramuscular injection (systematic treatment) (11). Systemic methotrexate induces higher maternal morbidity and mortality, as well as haematological systemic side effects such as thrombocytopenia and neutropenia. Nausea, vomiting and drowsiness are common side effects of methotrexate therapy(16).

Surgical treatment by suction, hysterectomy (17) or laparoscopic (18) excision of pregnancy may also be considered as alternative management options in CSP (19).

Local Administration of Methotrexate

The clinician carrying out the injection into the ectopic pregnancy requires experience in invasive guided ultrasound and diagnostic ultrasonography procedures (20). The methotrexate injection is given through the vaginal route with the patient under general anaesthesia and in the gynaecological position. Due to the risk of bladder injury during the procedure, a bladder evacuation with or without catheterization, must be performed just prior to the procedure. Using an endo-vaginal probe, and a marker on the ultrasound machine, the needle route is marked before the oocyte puncture needle is introduced and guided by transvaginal ultrasound into the gestational sac. Inside the sac, aspiration of gestational sac fluid takes place, ensuring that the needle is in the correct place. This is also done to reduce the risk of rupture due to increasing the volume inside the gestational sac secondary to the methotrexate injection. At the end of the procedure, the patient is checked for intraperitoneal haemorrhage via pelvic ultrasonography. On average, the procedure takes ten minutes, and it is important to monitor the patient after the procedure and follow up hCG levels (20).

Conclusion

CSP pregnancies are rare. Treatment may include conservative, medical or surgical treatments depending on the presentation and local expertise. Intragestational sac treatment with methotrexate is an effective method and avoids the surgical and anaesthetia-related risks and systemic side effects of methotrexate.

Declarations

Conflict of interest: N.A.

Ethical statement: Consent for publication from the patient was obtained.

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LITERATURE REVIEWS

Quantification of Haemoglobin A2 for mass screening of major haemoglobinopathies

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Abstract

Haemoglobinopathies are the most prevalent single-gene disorders and include a wide range of disorders most commonly thalassaemia and sickle cell disease (SCD). Multiple screening methods to diminish morbidly and mortality of those suffering with haemoglobinopathies have been developed including HPLC and ion-exchange microcolumn chromatography. Due to significant shortcomings upon use of these techniques, many studies in search of better screening strategies to quantifying HbA_2 levels in mass populations screening have been conducted. The aim of this review is to compare and contrast three methods for quantifying HbA2 to ultimately find a more effective protocol than standard methods for mass screening of major haemoglobinopathies. Alternative HbA_2 quantification protocols for mass screening established by Shihabi *et al.,* capillary zone electrophoresis (CE) in arginine-Tris buffer, Daniel *et al.,* MS/MS, *and* Kuntaruk *et al*., sandwich ELISA, were analysed and compared. Upon comparison, differences in interfering factors which effect successful separation of haemoglobin and quantification of HbA_2 were noted whereby sandwich ELISA was deemed better than CE and MS/MS for quantifying HbA_2 due to its high specificity. Moreover, comparison of the techniques with standard methods of HPLC and micro-column chromatography noted that both MS/MS and CE are better options for detecting Hb Lepore when compared to sandwich ELISA. To conclude, since ideally the selected method must be applicable for both neonatal and adult screening, then the most effective protocol forscreening major haemoglobiopathies in mass populations is MS/MS.

Key words: Haemoglobinopathies, HPLC, Chromatography, ELISA, MS/MS

Introduction

Globally, the most prevalent single-gene disorders are haemoglobinopathies, which result in structurally abnormal haemoglobin molecules (1,2). The most common haemoglobinopathies include sickle cell disease (SCD) and thalassaemia (α-thalassaemia and β-thalassaemia) (3). Thalassaemia is an inherited autosomal recessive genetic disorder that arises from flaws in globin chain synthesis. As a result, a reduction (+) or complete absence (0) of one or multiple haemoglobin chains occurs in people suffering from the genetic disorder (4).

The disproportion in haemoglobin chain production results in the formation of excess homotetramerβ-globin and γ-globin chains in α-thalassaemia and extra α-globin chains in β-thalassaemia (5). As a result of the decrease in β-globin chains, an increase in haemoglobin A_2 (HbA_2) is observed in β-thalassaemia, in conjunction with a reduction in the expected mean corpuscular volume (MCV) (6,7).

Unlike haemoglobin A (HbA), HbA_2 is only found in small quantities in the red blood cells (RBCs) of normal adults and is composed of two α-globin and two δ-globin chains. Since the level of HbA_2 expressed specifically in β-thalassaemia heterozygotes is increased, HbA_2 is reported to be the most predominant parameter used to identify β-thalassaemia carriers and screen for β-thalassaemia in adults (4,6). However, HbA_2 levels in individuals who are homozygous for β-thalassaemia provide no diagnostic value when compared to heterozygous individuals (8).

In both β+ and β0 heterozygotes, the increase in HbA_2 levels has been correlated with two molecular mechanisms. The first mechanism takes into account the events that lead to β-globinchain deficiency, which allows the α-globin chains to bind more readily to the δ-globin chains. On the other hand, the second mechanism considers the potential presence of β-globin gene mutations in the gene's promoter region. As a result, a decrease in transcription factor binding affinity occurs allowing the transcription factors to bind to the δ-globin gene promoter region. Simultaneously, the δ-globin gene transcription increases (4).

Moreover, an increase in HbA_2 concentration has also been observed in other haemoglobinopathies such as megaloblastic anemia in the absence of β-thalassaemia and haemoglobin S (HbS) trait (sickle- cell trait) (7,8).

In order to diminish the number of affected births and reduce childhood morbidity and mortality, especially in SCD and thalassaemia, multiple screening strategies have been developed (2). In fact, many laboratory protocols used to quantify HbA_2 are routinely performed in most hospitals around the world as part of the population screening for these genetic disorders (4). Population screening for haemoglobinopathies was dramatically transformed when automated high performance liquid chromatography (HPLC) packages were introduced (6).

At present, HPLC together with ion-exchange microcolumn chromatography, is still the standard protocol of choice for routine screening in most laboratories (4,5). In some countries, HPLC is the only protocol approved for adult screening whilst isoelectric focusing and HPLC are the protocols approved for newborn blood spot screening of thalassemia major (β0/β0 genotype) and SCD (6). Nonetheless, the HPLC protocol has significant shortcomings including its exorbitant cost and its inability to quantify HbA_2 levels in HbE-bearing subjects. Therefore, HPLC may not be the ideal protocol for quantifying HbA_2 levels in mass populations screening (4). As a result, many studies in search of better screening strategies have been conducted included those conducted by Shihabi *et al.* (2000), Daniel *et al*. (2007) and Kuntaruk *et al.* (2010).

Alternative HbA_2 quantification protocols for mass screening

In 2000, Shihabi *et al.* conducted a study to demonstrate the efficiency of capillary zone electrophoresis (CE) in arginine-Tris buffer at separating the major Hb variants HbC, HbS, HbA, HbF and HbA, and the ability to accurately quantify HbA_2 in sickle cell trait (7).

In their study, RBCs were collected in EDTA tubes, mixed with a haemolysis solution containing Tris buffer and injected hydrodynamical in a 33 x 50 mm capillary tube for 7 s. Following this, the sample was electrophoresed for 10 min at 9kV and 15 min at 6kV for qualitative separation and quantitation of HbA_2 respectively. The resultant data was then collected using the 'Peak Simple'
software.

As technology advanced, in 2007, Daniel *et al.* conducted a study aimed at determining theusefulness of the δ:β-globin peptide ratio as a substitute biomarker of HbA_2 levels in the identification of Hb Lepore and β-thalassaemia (6). Prior to this study, Daniel *et al.* had published a study using a simple peptide-based approach involving tryptic digestion of whole blood and multiple reaction monitoring (MRM) targeting peptide mutations for tandem mass spectrometry (MS/MS) screening of major haemoglobinopathies. These clinically significant haemoglobinopathies included HbE, HbS, HbC, HbOArab and HbDPunjab (8). The method used was highly specific and faster than standard methods, fulfilling all the requirements for newborn screening. In spite of this, since it could not be used to detect β-thalassaemia carriers, this method did not satisfy the requirements for adult screening (6).

Consequent to their 2005 study, in 2007 Daniel *et al*. added further MRM acquisitions in order to prove the MS/MS's quantitative potential in detecting β-thalassaemia. Additionally, they hypothesised that the proportion of β and δ-globins should correspond to the proportion of HbA_2, since HbA_2 has a tetrameric structure composed of equivalent α and δ-globins. In doing so, they
suggested that measuring δ peptide proteins by MS/MS may bring forth a useful substitute biomarker for HbA_2 and prove MS/MS to be a potential candidate for quantifying HbA_2 in β- thalassaemia (6).

In order to achieve their aims, Daniel *et al.* studied 150 blood samples with raised HbA_2, 163 with normal HbA_2, 8 with Hb Lepore and 43 with δ-globin chain variants, all of which were analysed by HPLC. To conduct MS/MS with flow injection analyses, all blood samples were incubated at 37 °C with trypsin for 30 min. Furthermore, MRMs for the β-globin (T2, T3, and T13) and δ-globin (T2, T3, and T14) tryptic peptides were obtained for 60 s. Following this, δ:β-globin peptide ratios were estimated. Finally, MS/MS and HPLC were used to investigate 26 paired dried blood spot and whole blood samples following storage for 1, 8, and 29 days (6).

Similarly, in 2010, Kuntaruk *et al.* conducted a study aimed at developing a sandwich enzyme-linked immunosorbent assay (ELISA) for quantification of HbA_2 for the detection of β-thalassaemia carriers which can then be adapted for mass population screening of β-thalassaemia in specific areas (4).

To achieve this, Kuntaruk et al. coated an ELISA plate with anti-HbA_2 monoclonal antibodies (mAbs) ThalA2-1 to capture HbA_2 present within the hemolysate sample. In this case, the hemolysate samples were prepared from RBCs collected from healthy adults and umbilical cords. Following this, a detector in the form of a second anti-HbA_2 mAb ThalA2-2 labelled with fluorescent dye (FITC) was used to identify the captured HbA_2. HRP-conjugated anti-FITC antibody was then used to detect the FITC-anti-HbA_2 mAb. As a result, a standard curve for the quantification of HbA_2 was established and the percentage of HbA_2 calculated was retrieved (4).

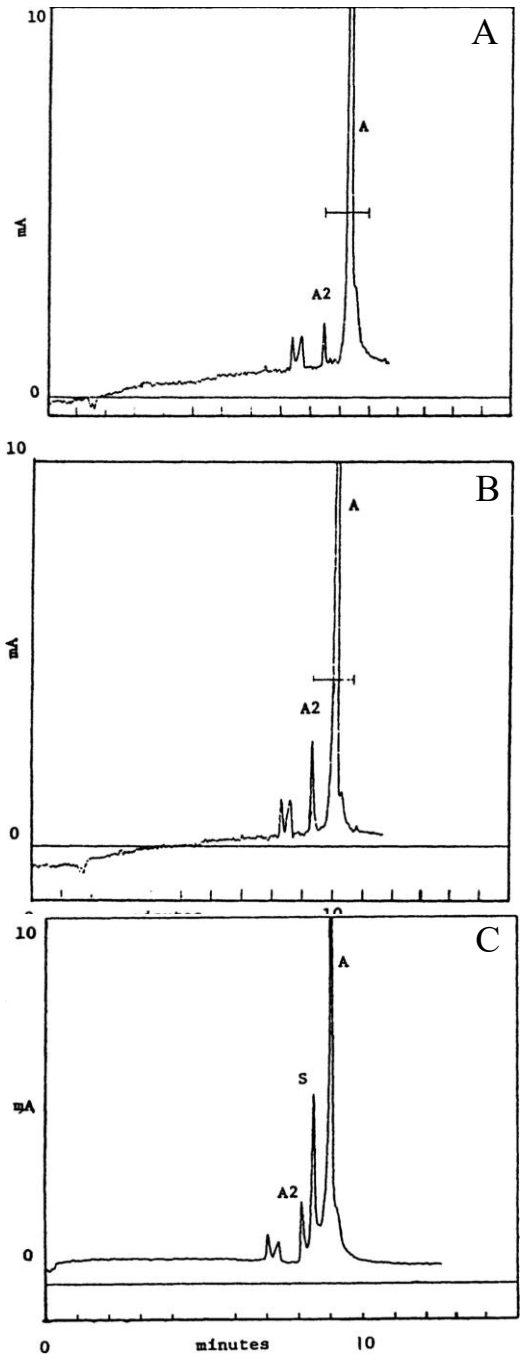
Finally, in order to assess the reliability of HbA_2 quantification using sandwich ELISA, a reliability analysis was conducted in 414 subjects already diagnosed with thalassaemia. This was carried out by considering the HbA_2 and MCV levels obtained using the standard HPLC method.

From the 414 subjects, 312 were non-β-thalassaemia heterozygotes comprised of 127 with HbE trait (heterozygous), 13 homozygous HbE, 18 suspect α-thalassaemia trait and 154 normal individuals. The remaining 102 subjects were β-thalassaemia heterozygotes (4).

Interfering factors to the effective separation of haemoglobin and quantification of HbA_2

In order to ascertain which method effectively separates and quantifies HbA_2 best, the results obtained from each study need to be thoroughly examined. Shihabi *et al.* reported that the major haemoglobins, including HbF, HbS, HbC and HbA separated well from one another especially when using the CE method. However, CE functions in the same way as many chromatographic and electrophoretic protocols since HbA_2 was seen to co-migrate and co-elute with HbC. Additionally, in a similar way to capillary zone agarose electrophoresis,HbE was also seen to co-migrate and co-elute with HbC and HbA_2 (7). As a result, HbA_2 may not be as accurately quantified with CE when compared with other methods.

In this study, Shihabi *et al.* obtained a good separation between all peaks of the HbA_2 results from individualswith β-thalassaemia, sickle cell trait and with no haemoglobinopathies (Figure 1) (7). Moreover, each case was distinct from the other since normal individuals had a normal HbA_2 peak, individuals with β-thalassaemia had a higher HbA_2 peak whilst individuals with sickle cell trait had an HbS peak. As a result, CE is a more suitable method for quantifying HbA_2 in sickle cell trait than the standard method micro-column chromatography since HbS did not interfere with HbA_2 and an HbS peakwas detected in those individuals with HbS trait.



Daniel *et al*. reported that MS/MS has the ability to accurately quantify and detect HbA_2 even in the presence of interfering factors including HbE and HbS, which respectively co-elute with HbA_2 and give falsely increased HbA_2 values (6). As a result, it can be assumed that MS/MS is a better method than CE for accurately quantifying HbA_2, since the former does not have interfering co-eluting factors.

Figure 1: HbA2 results from (A) an individual with no resultant haemoglobiophaties; (B) an individual with β-thalassaemia; and, (C) low MCV in an individual with sickle cell trait. Modified from Shihabi *et al.,* 2000.

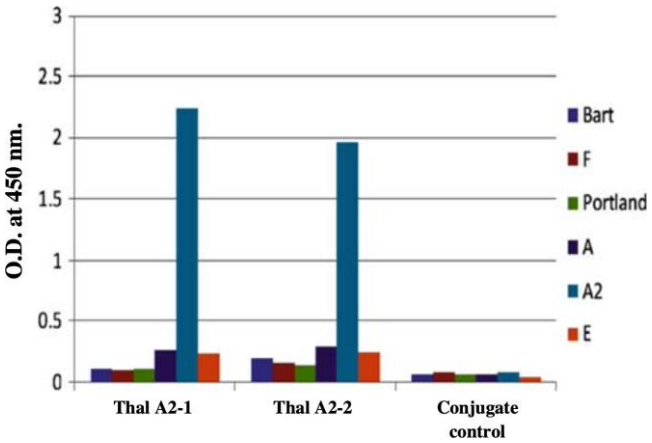
Furthermore, in the study conducted by Kuntaruk *et al.*, a good quantification of HbA_2 wasobtained and both ThalA2-1 and ThalA2-2 were seen to specifically react with HbA_2 (Figure 2) (4). These results demonstrate that sandwich ELISA can accurately quantify HbA_2 without being interfered with by confounding factors. This is because both mAbs are highly specific and bind to HbA_2 only.

Figure 2: Specificity of mAbs ThalA2-1 and ThalA2-2 to HbA_2. Retrieved from Kuntaruk *et al.*, 2010.

However, although HbC, HbS and HbE were not seen to act as interfering factors, δ-globin chain variants with abnormal δ-globin chains resulting from point mutations (ex:Hb Sphakia ($a_2d^{2(His- Arg)}$) may interfere with the sandwich ELISA by preventing the mAbs from binding specifically to the HbA_2 which may result in false negative results. As a result, particular care needs to be taken when interpreting results obtained from geographical regions where these abnormal δ-globin chains are common, such as European countries (4).

From the above it can be concluded that, sandwich ELISA is a better method than CE and MS/MS for quantifying HbA_2 due to its high specificity. However, due to the potential interfering mutations,MS/MS may be a better option in countries where the abnormal haemoglobins are highly prevalent.

Comparison with the standard methods of HPLC and micro-column chromatography



Moreover, when applying the sandwich ELISA for the quantification of HbA_2 levels in 112 blood samples, the sandwich ELISA demonstrated to have a comparable capability of detecting β- thalassaemia heterozygotes to that of HPLC with 95% accuracy, 100% sensitivity and 95% specificity (Table 1) (4).

Despite the ability to distinguish β-thalassaemia heterozygotes from non-β-thalassaemia heterozygotes and detect β-thalassaemia heterozygotes with almost identical accuracy as HPLC, lower HbA_2% were obtained in all subject groups when using the sandwich ELISA than HPLC. Additionally, although most samples tested were distinctly correlated, a few sampled showeda discrepancy in HbA_2 levels between HPLC and the sandwich ELISA (4).

Complimentary to Kuntaruk *et al.*'s study, Daniel *et al*. also demonstrated that MS/MS is a valid approach for HbA_2 quantification since the percentage δ-globin chain ratios were seen to highly correlate with the HPLC HbA_2 quantification measurements (Figure 3). Nevertheless, as was observed in Kuntaruk *et al.*'s study, the MS/MS peptide results were not completely alike with thoseof HPLC even after calibrating them against the WHO International HbA_2 reference reagent.

However, since the MS/MS results for each peptide were highly consistent and normal HbA_2 levels were successfully distinguished from increased levels, MS/MS still demonstrated to be a valid approach for HbA_2 quantification in β-thalassaemia (6).

Although HPLC was not mentioned in Shihabi *et al.*'s study, the alternative standard method micro-column chromatography was used instead for comparison purposes. As mentioned earlier, through Shihabi *et al.*'s results, it was proven that CE is a more suited method for quantifying HbA_2 in sickle cell trait than micro-column chromatography (7). However, similar to HPLC, micro-column chromatography can be effectively replaced for the quantification of HbA_2 and detection of haemoglobinophaties.

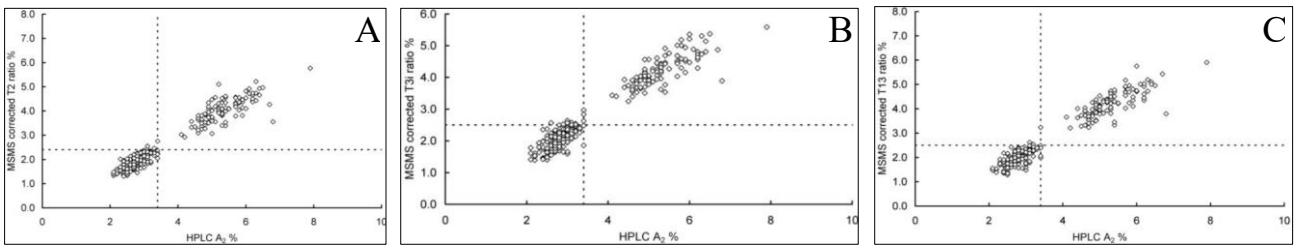
As a matter of fact, HbA_2 levels in individuals bearing HbE (homozygous HbE and HbE trait) were able to be quantified using sandwich ELISA whilst those individuals who were homozygous HbE could not. This was due to the fact that HbE did not co-migrate or co-elute with HbA_2 allowing accurate quantification of HbA_2 (4). Since neither HPLC nor micro-column chromatography are able to quantify HbA_2 in HbE-bearing subjects, the sandwich ELISA technique is a better option for mass screening of haemoglobinopathies than the standard methods used. Nevertheless, sandwich ELISA is not the sole method for quantification of HbA_2 in HbE-bearing individuals since this phenomenon was also observed when using CE (4). That aside, MS/MS was not observed to have the ability to quantify HbA_2 in these patients, making CE and sandwich ELISA both better candidates.

Another important haemoglobinopathy mentioned in both Kuntaruk *et al.* and Daniel *et al.*'s studies, was Hb Lepore, a δ-β-globin chain hybrid. In Daniel *et al.*'s study, it was argued that, since the mostcommon δ-globin chain mutation occurs in the T2 peptide and is clinically silent, the T2 peptide should not have been included. However, the T2 peptide is crucial for identifying three

Table 1: Accuracy, specificity and selectivity of the sandwich ELISA compared to HPLC fordiagnosing β-thalassaemia. Retrieved from Kuntaruk *et al,* 2010.

HPLC	ELISA		Total
	β-Thalassemia trait	Non-*β*-Thalassemia trait[a]	
β-Thalassemia trait	14	0	14
Non-*β*-thalassemia trait[a]	5	93	98
Total	19	93	112
Sensitivity	100% (14/14)		
Specificity	95% (93/98)		
Accuracy	95% (107/112)		
Positive predictive value (PPV)	82.3% (14/17)[b]		
Negative predictive value (NPV)	100% (93/93)		

Figure 3: MS/MS corrected (A) T2, (B) T3 and (C) T13 δ-β peptide ratio contrasted with HPLC HbA_2. Modified from Kuntaruk *et al.,* 2010.



reported Hb Lepore variants; Hb Lepore Baltimore, Hb Lepore Boston-Washington and Hb Lepore Hollandia (6). As a matter of fact, an increase in proportion of δ-globin chain peptides was predicted before the fusion point which was later seen as a 12-17% increase in the T2 peptide. This diagnostic pattern of an Hb Lepore, as reported by Rai *et al.,* may also be obtained using MS/MS (9).

On the contrary, Kuntaruk *et al.* reported that falsely high HbA2 levels may be obtained when usingthe sandwich ELISA method. As a result, Kuntaruk *et al.* argued that when dealing with higher thanusual levels of HbA2, other methods including HPLC and CE should be carried out instead (4). From this, it can be concluded that both MS/MS and CE are better options for detecting Hb Lepore when compared to sandwich ELISA.

Conclusion

After comparing and contrasting capillary zone electrophoresis, mass spectrometry and sandwich ELISA, the most effective protocol for mass screening major haemoglobiopathies is dependent on two main aspects. When comparing the methods for how accurately HbA_2 can be quantified, sandwich ELISA is the best approach since the method has high specificity towards HbA_2 and results proved that it is a suitable method for mass population screening of major haemoglobiopathies such as β-thalassaemia. Moreover, in comparison to standard methods, the best method for quantification and identification of haemoglobinpathies would be either MS/MS or sandwich ELISA since they are both better options when compared to standard methods. Since ideally the selected method must be applicable for both neonatal and adult screening, then the most effective protocol for screening major haemoglobiopathies in mass populations is MS/MS.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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The basal ganglia and its role in Parkinson's disease.

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Abstract

The basal ganglia consist of a collection of subcortical nuclei that play an essential role in the control of motor function but also too in cognition. These nuclei can in turn be divided according to their projections into input, output and intrinsic nuclei. The classical basal ganglia model was developed to explain the circuits within this structure. It consists of a direct pathway that promotes movement, an indirect pathway that inhibits movement and a dopaminergic pathway that modulates movement. Disorders are seen to arise due to a dysfunction in a part of the circuit. Such can be arranged in a spectrum that consists of hyperkinetic disorders on one side and hypokinetic disorders on the other. By identifying which parts of the circuit is dysregulated, one would be able to design therapies to elevate these symptoms.

Keywords: Basal ganglia, Parkinson's disease, movement disorders

Introduction

The basal ganglia can be defined as a series of nuclei that are located deep within the telencephalon (1,2). They are primarily involved in voluntary motor control. This collection of structures are also seen to be involved in other processes, such as those motivational, cognitive and emotional that eventually lead to movement being carried out. (3,4). In fact the associate, limbic, motor and orbitofrontal cortico- basal ganglia-thalamo-cortical loops have all been identified. For the purpose of this review emphasis will be made on the motor cortico-basal ganglia-thalamo-cortical loop, due to it being the most relevant in motor pathophysiology (5).

The classical model of Parkinson's disease

The classic basal ganglia model was developed in 1980's and is based on the cortico- basal ganglia- thalamo- cortical loop. It can be simplified as follow; projections arise from the motor and association cortex and terminate on the striatum (Figure 1). From here, impulses move to the pallidum that is the internal segment of the Globus pallidus (GPi) and the substantia nigra pars recticularis (SNpr). These in turn project onto the thalamus which then sends neurons back to the motor cortex (3). This model states that cortical activation, as a result of the intention to carry out a desired movement, leads to glutamate release on the striatal neurons. This results in the activation of the medium spiny neurons (MSN). Being inhibitory due to

GABA release, MSN result in SNpr and the GPi deactivation. Since now the SNpr and GPi have been inhibited, the GABA release from the SNpr and GPi is reduced and thus inhibition of subsequent structures, which include the thalamus, is lost. This allows for activation of venterolateral and venteroanterior thalamus. Hence these neurons can now activate the primary motor cortex, the premotor and supplementary motor cortex. This is known as the direct pathway. Furthermore, MSNs that project from the striatum to the pallidum also project onto the external segment of the Globus pallidus (GPe) via the indirect pathway. MSNs release GABA onto the GPe and result in its deactivation. Hence now the subthalamic nucleus (STN) which was previously inhibited by the GPe will be activated. This leads to the activation of the GPi because of glutamate release from the STN. The STN also activates the SNpr through glutamate release. Both the SNpr and the GPi contain inhibitory GABA releasing neurons, hence once activated they will inhibit the superior colliculus and the thalamic nuclei respectively. As a result, excitatory impulses arising from the thalamus and projecting to the motor cortex are abolished. Hence, the thalamus will no longer be able to stimulate the cortex and thus movement is revoked. (6-8) The direct and indirect pathway are theorised to work simultaneously so as to select intended movement and supress the undesired ones (9). This model also states that dopamine released from the substantia nigra pars compacta (SNpc) plays a major role in providing modulation of the inputs of the cortex from the striatum. The modulation depends on whether dopamine binds to D1 receptors located on neurons participating in the direct pathway causing excitation or to D2 receptors located on neurons in the indirect pathway, causing inhibition (10).

It should be noted that the STN receives additional projections that arise directly from the cerebral cortex, these are excitatory impulses that form part of the hyperdirect pathway. Activation of this pathway leads to inhibition of the thalamus via the activation of the SNpr as previously described and hence no movement occurs (6)

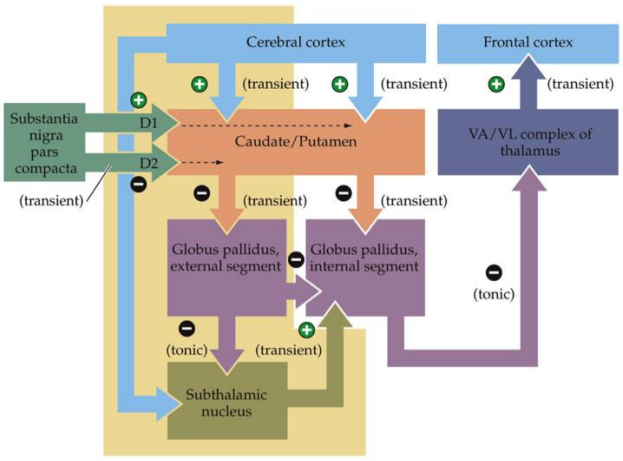


Figure 1: The classical basal ganglia model demonstrating the direct, indirect pathway and hyper-direct pathways. Retrieved from White *et al.,* 2001.

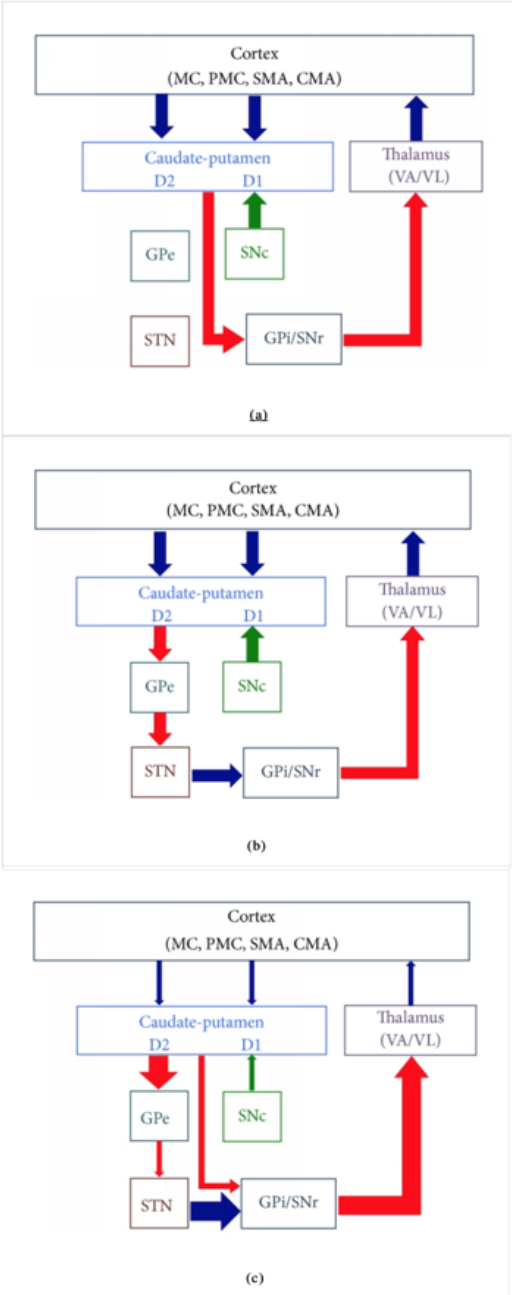
Overview of basal ganglia movement disorders

In terms of disorders that can arise, one can compose a spectrum with two opposing ends; hyperkinetic disorders, characterised by involuntary movement, like Huntington's disorder on one side and hypokinetic disorders, characterised by a diminished motor activity (excluding the resting tremor), like Parkinson's disease on the other side. It is suggested that involuntary movements seen in hyperkinetic disorders are a result of a diminished inhibitory output from the basal ganglia. This results in a decreased suppression of fronto-cortical areas which in turn initiate movement. The reversal of this is observed in hypokinetic disorders (11).

Parkinson's disease

Parkinson's disease (PD) collectively refers to neurodegenerative conditions that affect different parts of the brain. Parkinsonism in turn refers to the motor impairments experienced such as akinesia, bradykinesia, rigidity and resting tremors. These motor impairments are largely attributed to a diminished dopamine in the basal ganglia. It has been shown that parkinsonism is a complex connectivity disorder that arises as a result of dysfunctional activity in a group of neurons mainly in substantia nigra. These neurons in turn alter the synchrony, excitation, oscillatory activity and also sensory response of regions in the cerebral cortex that are involved in movement planning and execution. Signs, including both motor and non-motor will start to appear once substantial loss of nigrostriatal neurons has taken place. (12)

Degeneration of the dopaminergic neurons in the SNpc is observed, while some of these neurons take decades to degenerate, neurons that project to the putamen do so at a faster rate. (12). Dopamine loss results in the reduction in post synaptic potential facilitation in the direct pathway and a reduction in the post synaptic potential inhibition in the indirect pathway, allowing for the latter to take over (Figure 2). This results in an abnormally high inhibition arising from the basal ganglia reducing the thalamic activation of upper motor neurons within the cortex hence resulting in the hypokinesia experienced (6). Thus, dopaminergic neuron loss leads to GPe neurons firing less and GPi, STN and SNpr cells with a greater spontaneous discharge and increased response to cortical stimulation. Studies have shown that there is also a change in their firing patterns as well as firing rates. An increased tendency to fire in a burst of action potentials was observed in the striatum and STN as well as enhanced



rhythmic/oscillatory activity. In addition, there was also increased synchrony between neighbouring neurons. Burst activity of the Globus pallidus and STN was seen to be more prolonged **Figure 1:** A simple representation of the direct pathway in (a), indirect pathway in (b) and the alteration observed in Parkinson's Disease (**blue arrows**: excitatory glutaminergic pathways; **red arrows**: inhibitory Gabaergic pathways; **green arrows**: dopaminergic pathways). Retrieved from Magrinelli *et al.,* 2016.

in terms of its duration, as well as in the length of the individual burst (Figure 3). The

presence of abnormal oscillatory activity within each nucleus and among structures in Parkinson's disease has also been recognised. This may in turn contribute to the development of parkinsonism. This is because the oscillatory activity in a single cell in the basal ganglia is reflected in the cerebral cortex. (12,15).

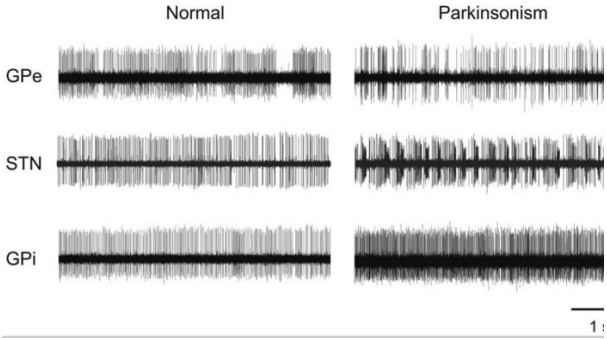


Figure 3: *A figure that illustrates the change in single cell activity as observed in normal and MPTP treated monkeys (to represent parkinsonian patients). Each segment represents a 5s duration. Retreived from Galvan and Wichmann, 2008.*

In addition to this, dopamine loss also results in other secondary changes such as the reduction in dendritic spine density of the MSNs, especially those present in the putamen. This would have major pathophysiological significance as it results in major alterations in the corticostraital and the thalamostriatal projections as it may account for diminished glutamatergic input (Figure 4). Studies have shown that D2 receptor expressing MSNs are affected to a greater extent and loss of spines may be a result of calcium channel dysregulation. Furthermore, dopamine reduction also leads to changes in both sensitivity and density of dopamine receptors (12,15). A study was conducted by by Gerfen and his colleagues using rats with lesions nigrostriatal dopamine pathway induced by 6-hydroxydopamine to serve as Parkinson's disease models. The latter showed that D1 receptor mRNA was reduced in the MSNs participating in the direct pathway and D2 receptor mRNA was increased in MSNs participating in the indirect pathway (16).

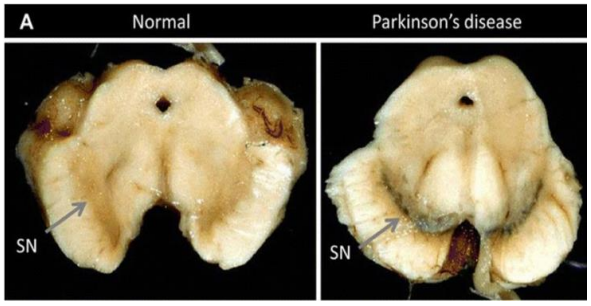


Figure 4: Anatomical changes observed a result of substantia nigra (SN) dopaminergic neuron degeneration Retrieved from Mandel *et al.,* 2010.

Since the pathophysiology underlying Parkinson's disease is dopamine depletion, indicated treatment is to provide pharmaceutical products that replace this dopamine loss. In fact, L- DOPA (levodopa) a dopamine precursor has been shown to be quite successful and more to the point, is considered to be the gold standard for PD patients. This precursor encourages dopamine synthesis by midbrain neurons (17). Additional drugs can be added to L – DOPA so as to increase L- DOPA's half-life, hence allowing for a reduced dose to be prescribed. An example of the latter is Carbidopa, a dopamine decarboxylase inhibitor. This block peripheral breakdown of L- DOPA, as it is unable to cross the blood brain barrier. Hence, increasing the amount of L- DOPA delivered to the brain, without an increase in L- DOPA dose (18). None the less, some patients also experienced dramatic side effects when taking L- DOPA like emotional disturbances and dyskinesia (17). In fact, L-DOPA dyskinesia is one of the most denilitating side-efects faced by patients suffering from this disease who are taking this particular treatment. This occurs when antiparkinsonian effects of this drug are maximal (19). The on and off phenomenon can be used to describe the effects of L- DOPA after long term use. In the off-phase, patients experience negative symptoms such as rigidity due to the lack of drug efficacy. In contrast, the on phase describes positive symptoms which can range from normal

movement to abnormal movement. Sometimes the term intermediate is used to describe normal movement (24). This phenomenon is thought to arise due to pulsatile administration of L –DOPA (23). This leads to an increased gene expression of the neurotransmitter in the direct pathway while failing to normalise the pathogenic upregulation of gene expression in the indirect pathway .Recent studies have indicated that dyskinesia experienced with this treatment is a result of these neurotransmitter changes (20,21).

Another way of targeting this disease is by increasing the activity of the ventrolateral and ventroanterior nuclei of the thalamus. This can be done by reducing the activity of the GPi. In addition, one can also aim to reduce STN activity which would also result in a reduction in GPi impulse generation. This can be done through deep brain stimulation, a recent advancement of neuro-medicine in the last two decades that involves the delivery of adjustable stimulation which in turn can offer a therapeutic effect for disorders related to an abnormal circuitry, which allows for neuromodulation of these targets described. This technique is actually applied to modulate the activity of the STN and GPi that are hyper activated. Improvements in the quality of life of patients that underwent this procedure were reported even in those with advanced PD. When deep brain stimulation is used along with L-DOPA treatment, potentiation of their beneficial outcomes occurs resulting in decreased PD symptoms (17,24).

PD arises from dopamine producing cell degeneration in the substantia nigra pars compacta, hence replacement of these cells can also be a way of increasing dopamine production. Treatments have been developed to target this by providing nigral cell transplantation. This method utilises embryonic midbrain neurons which are then transplanted into adult brains. These neurons are then able to form axons in the scar tissue present resulting in the innervation to be resorted (17). A somewhat similar approach was also devised using genetically modified cells that contain the necessary genes to produces tyrosine hydroxylase, an enzyme utilised in the dopamine production (6).

Conclusion

Despite information with regards to the core structure within the basal ganglia being available for some years, the actual role of these structures as well as its projections to both cortical and subcortical structures is still in a process of development, with more circuits both intrinsic and extrinsic being discovered. Thus, more research is required in this grey area. This allows for the ability to link clinical observations to those modelled in the laboratory to be able to devise new treatments hence potentially increasing the life expectancy and quality of life of patients who have both physical and cognitive impairments as a result of a dysfunction in this major core structure.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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Interleukin-1 and Inflammation in Cardiovascular Disease

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Abstract

Interleukin 1 (IL-1) is a pro-inflammatory cytokine with important roles in innate immunity and tissue homeostasis. Inflammation is crucial as it protects the host by reacting against pathogens and repairing tissues however, improper use of the inflammatory cascade results in pathogenesis of several acute and chronic diseases. IL-1 together with the NLR family pyrin domain containing 3 (NLRP3) inflammasome are key players in inflammation and modulate cardiac function as well. Literature concerning the role of IL-1 and the inflammasome is reviewed, together with a concise narrative overview pathways affected during signaling. Specific examples of the role of IL-1 in pathogenic outcomes in cardiovascular disease (CVD) are also reviewed.

Key words: Interleukin-1, cardiovascular diseases, inflammation, atherosclerosis, myocardial infarction, arrythmias,

Introduction

Interleukin-1 (IL-1) is a pro-inflammatory cytokine assembled by the innate immune system. It acts on innate immune cells as well as on lymphocytes during adaptive immune responses, for instance, naïve T-helper cells can be differentiated and maintained with the help of IL-1 (1).

IL-1 was initially referred to as an endogenous pyrogen since it increases body temperature, resulting in pyrexia which is the hallmark of inflammation. The IL-1 family consists of 11 members and 10 receptors, of which IL-1α and IL-1β are the most commonly studied. IL-1 receptor antagonist (IL-1RA) regulates their activity since it binds to the IL-1 receptor instead of IL-1α and IL-1β (2). The IL-1 receptor is a heterodimer made up of IL-1Ra and IL-1R accessory protein (IL-1RacP) sub-units. IL-1α or IL-1β binding with the IL-1 receptor induce the synthesis acute phase and pro-inflammatory proteins a via a signal transduction cascade, contributing to the inflammatory response.

Most of the IL-1 family members act indirectly on immune processes. Activation of nuclear factor kappa light chain enhancer of activated B cells NF-κB, IL-1β directs transcription and gene expression of cyclooxygenase type 2 (COX-2), type 2 phospholipase A and inducible nitric oxide synthase (iNOS), resulting in the production of prostaglandin-E2 (PGE2), platelet

activating factor (PAF) and nitric oxide (NO). Thus, the biological effects of IL-1, mediated through the aforementioned signal transduction products include: pyrexia, diminished pain threshold, vasodilatation and lowered blood pressure. NO and PGE2 also significantly affect immune responses, such that PGE2 non-specific T-cell suppression is highly prevalent (4). Moreover, IL-1 signalling modulates reparative processes through regulation of gene expression in fibroblasts and smooth muscle cells, achieved by altering the Matrix Metalloproteinase/Tissue Inhibitor of Metalloproteinases ratio (5).

IL-1β signalling induces release of secondary pro-inflammatory cytokines and chemokines including IL-6, tumour necrosis factor α (TNFα) , and granulocyte colony stimulating factor (G-CSF), which provide for the transient development of the inflammatory disease (6). IL-1β is a powerful pro-inflammatory cytokine which is activated via two stages: IL-1β precursor is synthesised consequent Toll-like Receptors (TLRs) activation. Then, pro-IL-1β is transformed to mature IL-1β with the aid of caspase-1 which activates pro-IL-1β by cleaving it. IL-1β causes vasodilation, recruitment of leukocytes through IL-8 and activation of neutrophils, leading to phagocytosis, degranulation and oxidative burst activity to occur (3).

Interleukin-1 in Atherosclerosis

Patients who have elevated inflammatory markers such as C-reactive protein (CRP), are at more risk of suffering from cardiovascular disease (CVD) even if otherwise healthy (7). In patients with a history of CVD, decreased C-reactive protein (CRP) levels result in a similar decrease in the incidence of CVD when compared to decreased LDL-cholesterol levels (8). In the past decades, atherosclerosis has been defined as a type of inflammatory disease. Pro-inflammatory lipoproteins running in the circulation increase the permeability of endothelial cells thus, more lipids and inflammatory macrophages (or foam cells) subside in the tunica intima of the blood vessels. Cholesterol plaque develops, which consists of a necrotic core and a fibrous cap (9). Over time, this deposition induces the production of cytokines including IL-1α, NLR family pyrin domain containing 3 (NLRP3) inflammasome activation, IL-1β and IL-18 (Figure 1). This deposition of fats and cholesterol clogs the vessels causing disruption in blood flow and is partly attributed to elevated expression of IL-1β and IL-1R in plaques and the more they are expressed, the more severe is the disease (10). IL-1 has various effects on cells involved in atherogenesis, mainly the endothelial cells, smooth muscle cells and macrophages.

When IL-1β acts on endothelial cells it enhances the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (11). IL-1β also activates chemokines such as monocyte chemoattractant protein-1 (MCP-1). This attracts phagocytes which are active mediators in CVDs. IL-1β further acts on smooth muscle cells by directing the production of platelet-derived growth factor which can direct smooth muscle cell proliferation (12). IL-1β also stimulates further plaque growth and rupture, which together factors contribute to atherosclerosis that may result in the formation atherothrombosis (13).. Necrosis and apoptosis are frequent events which occur in atherosclerotic lesions and these can activate IL-1α and IL-1β as well (14). IL-1 also induces activation and liberation of another noteworthy cytokine; IL-6. IL-6 stimulates hepatocytes which in turn increase the synthesis of fibrinogen which boosts

thrombosis and plasminogen activator inhibitor (PAI) which in turn restricts fibrinolysis.

IL-6 also directs hepatocytes to boost synthesis of C-reactive protein (CRP). Thus IL-1 amplifies the inflammatory response due to a single IL-1 molecule inducing multiple IL-6 molecules which further activate the expression of several pro-inflammatory mediators, further promoting atherogenesis and the development of several other CVDs (12).

It is essential to highlight the importance of the balance which needs to present between IL-1 and IL-1Ra. Nicklin *et al.* (2000) utilised a IL-1^{++}/IL-$1Ra^{--}$mouse model to investigate the effects of an imbalanced IL-1 and IL1-Ra ratio. Through this model, IL-1 was unopposed and as a consequence, the mice developed severe vascular inflammation together with the occurrence of several inflammatory cells across the wall of the arteries. The vessel walls were observed stenosed and collapsed leading to infarction (15). Other *in-vivo* rodent outline the atherogenic properties of both IL-1α and IL-1β. The first strain consisted of mice deficient in apolipoprotein E (ApoE). When the bone marrow of the first strain of mice was replaced by an IL-1α or IL-1β deficient one, these mice presented with reduced atheromas when compared to the control mice which received the wild type bone marrow (17). Notably, in this study, IL-1α deficient mice presented with greater protection against atheroma formation rather than when mice where deficient in both IL-1α and IL-1β. Thus, compound deficiency of both IL-1α and IL-1β, does not give additional protection in atherosclerotic lesion formation (18). The second strain consisted of mice lacking in low-density lipoprotein receptor (LDL-R) with overexpression of IL-1Ra. The mice in this strain presented with significant reduction in atheroma formation. ApoE deficient mice deficient in IL-1Ra, presented with reduced plaque formation in the root of the aorta but not in the brachiocephalic artery. Deficiency of IL-1Ra also resulted in differences in the structural remodelling of the endothelium and collagen resulting in the maintenance of the atheroma's fibrous cap (18). This may thus demonstrate IL-1Ra's role in protecting against atheromatous plaque rupture (19).

High cholesterol levels induce the IL-1β precursor to be cleaved to active IL-1β. This

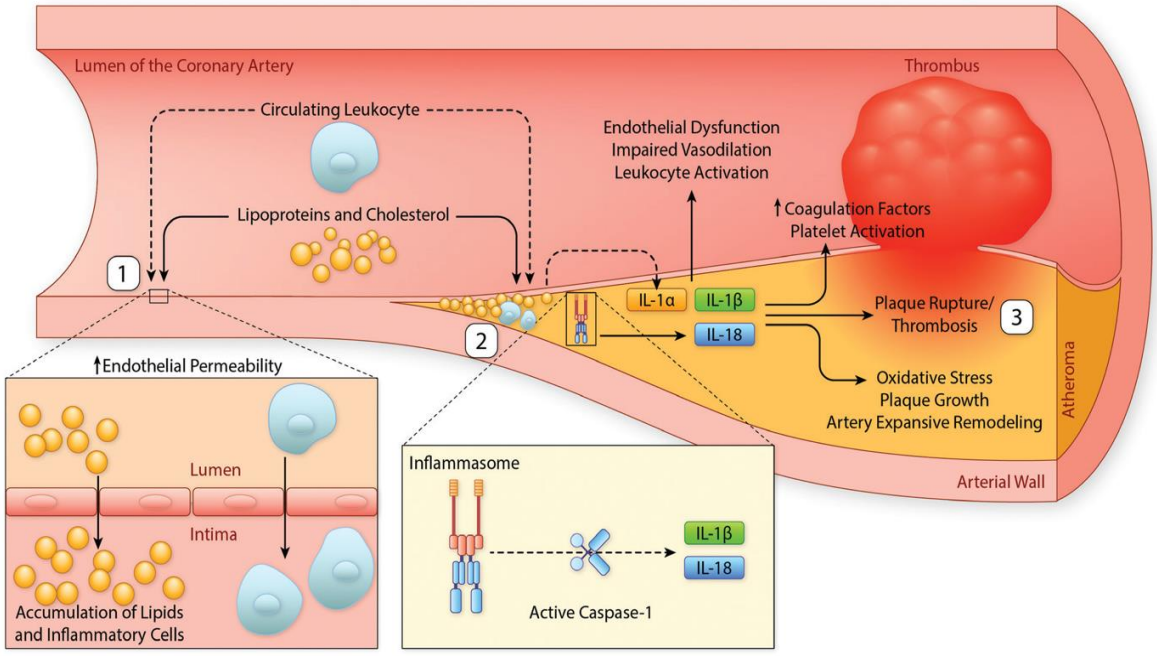

Figure 1: A visual representation of the role of IL-1 in the process of atherosclerosis. Retrieved from Abbate *et al.*, 2020.

occurs due to the deposition of cholesterol crystals in plaque which direct the activation of the NLRP3 inflammasome by activating cathepsin B in the cytoplasm (20). Indeed, experiments performed on LDL-R deficient mice with NLRP3, apoptosis-associated speck-like protein containing a CARD domain (ASC), IL-1α or IL-1β deficiency fed a high fat diet, resulted in a significant reduction of atherosclerotic lesions when compared to the mice with wild type bone marrow (21). Mice with an irregularly functioning inflammasome and caspase-1 deficiency coupled with reduced ApoE or LDL-R, were considered to be protected against inflammatory reactions when compared to mice that are ApoE or LDL-R deficient but have a functional caspase-1 (22). This demonstrated the crucial role of caspase-1 as a mediator in activating pro-IL-1β to IL-1β and further progression of inflammation. The extent to which NLRP3 inflammasome is expressed in plaque has been shown to correlate with the severity of the CVD (23). Another study however shows that ApoE mice with a dysfunctional NLRP3 inflammasome, does not decrease the severity or the incidence of atherogenesis (24). The reason for which these studies produced opposite results with regard to the role of the NLRP3 inflammasome in atherosclerosis still remains indefinite thus, further studies need to be implemented to rule out the proper role of the inflammasome.

Interleukin-1 in Myocardial Infarction

When the ventricular cardiomyocyte undergoes necrosis following an infarct, an inflammatory cascade occurs which functions to clear away the damaged tissue and replace it with fibrous tissue resulting in with scarring. Contents within the dying myocardial cells are released and an inflammatory response initiates via activation of the innate immune system. Receptors at the cell surface sense the released ligands as danger signals and activate cytokines and chemokine pathways (25). The NF-κB system is activated which causes endothelial cells to upregulate the expression of adhesion molecules. Chemokines bind to their receptors on circulating leukocytes and these infiltrating leukocytes remove the infarct composed of necrotic cells and debris (26). Consequently, cytokines and growth factors are induced which adjust extracellular matrix metabolism and activate fibroblasts and endothelial cells causing healing to shift from the inflammatory to the proliferative phase (27). Then leukocytes undergo apoptosis and are removed from the area whilst myocardial fibroblasts at the area of infarction differentiate into myofibroblasts which produce a vast amount of extracellular matrix proteins (28). New vessels start to form surrounded by a muscular coat whilst uncoated vessels start to regress. Inflammatory cells, fibroblasts and vascular cells undergo apoptosis resulting in mature scar formation made up of collagen (28). As healing is taking place, ventricular remodelling occurs. This includes the remodelling of both necrotic zone and non-infarcted zone of the ventricle. This may result in ventricular hypertrophy resulting in a deterioration of cardiac function (5). IL-1 family members are highly expressed in myocardial infarction and a clinical analysis confirms that serum IL-1β levels increased in patients with acute myocardial infarction (AMI) soon after chest pain sets in. However other studies did not record increased serum IL-1β levels in patients with AMI. The discrepancy in these aforementioned studies may have occurred since IL-1β may bind to large proteins such as α2 macroglobulin or to the IL-1R2 receptor. As a result, serum IL-1β levels could be harder to detect (5). IL-1Ra levels were also significant in AMI patients.

Indeed, IL-1Ra levels were significantly elevated in patients with AMI and IL-1Ra expression occurred ahead of release of necrosis markers. Such levels of IL-1Ra correspond with the degree of cardiomyocyte loss (29). IL-1α acts as a warning biomarker early on in the onset of AMI, and it activates IL-1β activity via activation of the NLRP3 inflammasome (30).

IL-1 signalling also induces the inflammatory response after infarction takes place. In IL-1Ra null mice, decreased neutrophil recruitment was observed together with enhanced neutrophil apoptosis. This was suggested to occur since because IL-1 has the ability to prolong neutrophil survival by preventing their apoptosis (31). IL-1 pro-inflammatory effects may intensify injury via several pathways. For instance, enhanced neutrophil levels may result in direct cardiomyocyte death and IL-1 signalling, which may activate matrix degradation thus enhanced matrix remodelling of the ventricle takes place. Thus, evidence in IL-1Ra deficient mice shows that IL-1 may contribute to injury, but it does not aggravate it (31).

IL-1 produces several effects on the myocardium: it induces apoptosis alone or alongside other cytokines such as interferon γ (IFN γ) and TNFα (5). This further promotes cardiac muscle injury. IL-1 causes cardiomyocyte hypertrophy by elevating expression of atrial natriuretic factor and repressing Ca^{2+} regulatory genes (32). IL-1β represses cardiac function as well via NO-dependent and NO-independent pathways and also via inhibiting c-adrenergic agonist which elevates cardiac contractility and cAMP accumulation. Apart from IL-1 leaving its effects directly on cardiomyocytes, it also influences gene expression of several cells which aid in infarct healing (Figure 2). IL-1 activates both endothelial cells and leukocytes. These induce adhesion molecule production and integrin expression respectively. Moreover, IL-1 also induces macrophages and endothelial cells to produce chemokines. These attract leukocytes to site of injury via chemotaxis. Fibroblasts are also affected by action of IL-1 and are involved in the reparative process. In IL-1Ra null mice, fibrosis following inafrct was significantly reduced (5); demonstrating the crucial effect IL-1 has on fibroblast activation and migration to the site of injury. Additionally,

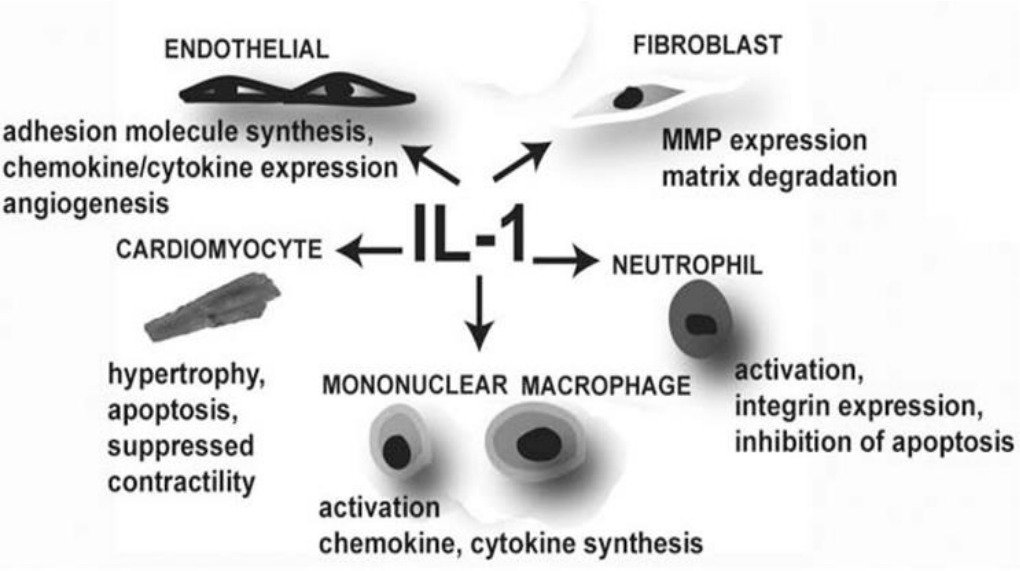

Figure 2: The effects of IL-1 on mediators of infarction and healing. Retrieved from Bujak and Frangogiannis *et al.*, 2009.

IL-1β enhances expression of angiotensin II type 2 receptors on cardiac fibroblasts, thus allowing for fibrous tissue build up (33). Transforming growth factor- β (TGF- β) is a pro-fibrotic mediator which was also reduced in infarcts conducted in IL-1Ra null mice, causing less collagen to be deposited in the healing scar and the area around the infarct (34).

IL-1 upregulates matrix metalloproteinase (MMP) synthesis, resulting in reduced collagen deposition and consequent matrix degradation (35). Suppression of the inflammatory response may be advantageous due to it reducing the extent of fibrosis of the infarcted ventricle. Collagen deposition and MMP expression are downregulated in IL-1Ra null infarcts thus interstitial remodelling is decreased (36). Finally, IL-1 enhances angiogenesis but the mechanism through which this is carried out is still unknown. This is shown by a study which involved three rat models that had undergone IL-1 inhibition in which new vessel formation was suppressed (37).

Interleukin-1 in Arrhythmias

Several studies performed on guinea pigs show that IL-1 is able to lengthen cardiomyocyte action potential (AP) via modifications in Ca^{2+} channels function (38). A recent study highlights the role of IL-1α in prolonging the action potential whereas IL-1β mediation resulted in extra-systolic patterns in rat atrial cardiomyocytes (39). Another model of diabetic mice outlines IL-1β acting in a similar fashion to IL-1α in lengthening the action potential and IL-1β also diminishes the K^{+} current and upregulates Ca^{2+} entry in cardiomyocytes. These IL-1β induced modifications are responsible for arrhythmia generation. These arrhythmias can be successfully eliminated by treatment with IL-1 receptor antagonist or by inhibiting the NLRP3 inflammasome (40). IL-1β is reported to inhibit L-type Ca^{2+} channels and inhibits expression of sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) and phospholamban (41). IL-1β also leaves its effect on connexin 43 (Cx43), which is a crucial protein present in gap junctions of cardiomyocytes and is a main contributor to the unity of the cardiomyocytes allowing them to beat in synchrony as a single unit (42). Thus cardiomyocytes may be disconnected from one another as a result of IL-1β Cx43 downregulation.

Interestingly, IL-1β is upregulated during atherosclerosis and AMI. Similarly, models of atherosclerotic mice post infarction showed a threefold elevation in IL-1β levels expressed, together with a twofold decrease in Cx43 thus resulting in a corresponding elevation in ventricular arrhythmias (43). In a case-controlled study in which 122 patients had atrial fibrillation and 63 patients acted as controls, it was noted that IL-1 levels were remarkably higher in atrial fibrillation (AF) patients thus showing the great role IL-1 plays in progression of arrhythmias and atrial fibrillation amongst other cardiovascular diseases previously mentioned (44).

Conclusion

The recognition of the part IL-1 plays in inflammation together with the discovery of specific external signals which trigger the inflammatory cascade, are crucial providers for the proper apprehension of the aetiology of several human diseases. This field of study is of increasing interest within the in the medical community . The biology of IL-1 has is polarised since it provides both beneficial and destructive contributions to patients' response to infection and injury. The IL-1 cytokine family together with the NLPR3 inflammasome are vital in responding to injury and are the key contributors to the

CVDs. Pre-clinical and clinical studies of IL-1β blockade have been demonstrated to be a promising approach in in the treatment of CVDs. Thus, IL-1β inhibition presents us with promising occasions where we can combat inflammatory diseases. Patients with risk of recurrent inflammation present with elevated CRP levels and benefit immensely from IL-1 blockade. Notably, the advancements made in this area of study were immense throughout the years, but numerous questions remain unanswered, and more studies need to be implemented. For instance, whether specific inhibition of exclusive cytokines or common receptors (e.g. IL-1Ra by anakinra which blocks both IL-1α and IL-1β) represent suitable therapies in CVD has yet to be confirmed. Nonetheless, we have entered an exciting era in which the clinical benefits from decades of research done to outline the role of immune and inflammatory pathways in CVDs can be consolidated.

Declarations

Conflict of interest: N.A.

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Apologies - A Necessary Soft Skill or Something to Avoid?

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Abstract

An apology can be thought of as a sincere expression of regret and emotional support, but can at times be interpreted as an admission of guilt. While countries have different laws relating to the topic of apologies in medical practice, the duty for open disclosure is being increasingly stressed upon medical practitioners. This narrative review seeks to discuss the definition, structure, role, effectiveness, and laws governing apologies in medical practice. It also aims to discuss if and how a physician should apologise and the practicality of apology laws in disclosing information to patients. Research highlights that fewer lawsuits result when apologies are issued by medical practitioners. The two main types of laws governing medical apologies are the tort system and the no fault system. Political lobbying and media emotionalization of certain cases influence lawmakers' decisions and so the implementation of these systems. While issuing an apology is effective, apology laws do not provide a definite positive impact in this regard. When a medic feels that an apology is warranted, they should discuss the full issue with a superior and peer, and take into consideration the local jurisprudence governing their profession. It can be displayed that successful training and teaching in the correct use and delivery of an apology increases the effectiveness of an apology when one is justified. This is many times lacking in undergraduate medical education.

Introduction

In a world where compassion is increasingly encouraged in healthcare, and where medical students are taught to empathise with patients, should a medical practitioner or a medical student apologise? How should one do this in terms of the healing process? What are the legal implications of doing so? Is there a different way of expressing regret, while not legally implying that one is at fault?

Although students are equipped with all the necessary medical knowledge to effectively treat diseases after finishing medical school, non-technical skills such as situational awareness and effective team communication are barely touched upon. Among these skills is the ability to address medical errors and give an appropriate apology (1).

In the original "apology," Plato provides a defence of his actions rather than expressing regret (2). Over the centuries, the definition of an apology has changed to an expression of

regret for causing trouble or hurting someone (3).

Due to the above definition, an apology is sometimes, and in some jurisdictions, taken as an admission of guilt, and thus carries a legal liability for the action by which a patient suffers harm (4). Yet, in most cases when a patient is harmed, both the patient and the physician desire an apology to express sympathy or to help in the therapeutic process. Thus the worry of creating a legal liability through an apology creates a feeling of a general lack of emotional support on both sides (5). This fear is not unfounded, as in the United States of America doctors on average face at least one malpractice lawsuit throughout their professional careers (6).

Due to this fear, so-called "apology laws" have been adopted by numerous nations and states to make physicians' expressions of regret inadmissible in the eyes of the law when disclosing medical errors and complications (7).

What constitutes an apology?

The Role of Apologies in Healthcare

During the medical care of patients, especially during medical error disclosure, malpractice, and issues of patient safety, apologies may be critical in supporting patients and their relatives. Giving a sincere apology is one of the most effective tools in doctor-patient communication and an important component of the patient-centred approach in healthcare (8).

An apology should be offered when an error has been made and harm has occurred, or there is potential for harm to occur. Yet, not all errors merit an apology. Misspelling a word in a doctor's note may not merit an apology, but misspelling similarly sounding medications might merit one. Similarly, the extent and severity of the error might merit a more profound apology, from several people or administrators representing the institution (9).

A Duty For Open Disclosure

Patients are owed the truth from their physicians. The process of disclosure of information on medical errors should be transparent, open, and honest (10). Many times patients express anger about the treatment after the error rather than because of the error itself. The patients and their relatives should get to know of the errors in a timely manner, and from the medical practitioners themselves, rather than an error known to the physicians being highlighted by the patients or relatives. As such an open, honest, and timely disclosure of the events leading up to, and following the error should be the only approach in cases of medical error (11).

How to Apologise

A good and effective apology is done at the right place, and at the right time (Figure 1). When one becomes aware of an error, the error should be reported immediately to the caring consultant. Breaking this information to the patient or family might be an emotional time, so this process should be slightly delayed until most facts are clear, to

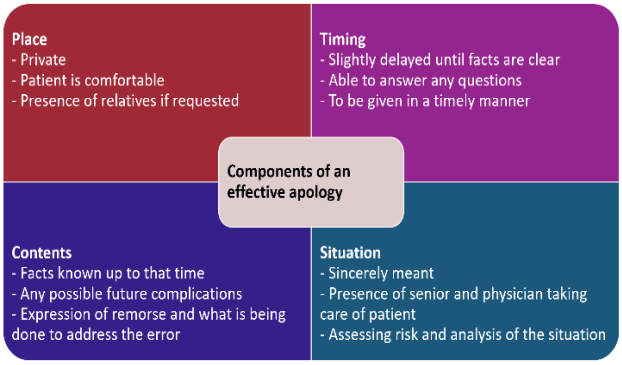

Figure 1: Illustrative Representation of What Makes an Effective Apology

avoid not being able to answer questions which might be asked. On the other hand, waiting too long might lead to the relatives or patients finding out through other means, and being accused of deception and disregard (9,12).

The place where this apology is issued should be somewhere private, and it should be made sure that the patient is comfortable. If support to break the news to relatives is requested by the patient, this should be adequately prepared in an environment of privacy (9,13).

The apology should be sincerely meant, and be tailored to the patient's situation. It should contain the facts known so far, possible complications, and what further studies one will make to assess the error. This should be done in the presence of the physician in charge of the patient, a charge nurse or peer, and in some cases a hospital administrator. The patient should also be informed of any support available to them and their relatives (13). Furthermore, this should not be a forced apology in which the institution obliges the responsible person to give an apology (14).

The formulation and structure of an apology differ according to the situation. The clinician should keep in mind the outcome of the error in choosing the way in which one expresses the apology. If the outcome is a development of an underlying medical condition, it is a good idea to offer an expression of concern and sympathy, albeit not an apology. If there is a risk of investigation or further treatment in the outcome itself, an expression of regret should be provided, but admittance of responsibility should not be provided in the form of an apology. If, after careful analysis of the situation, the outcome is determined to be related to a system or healthcare provider failure, an apology should be considered by the organisation or provider, as it is appropriate and expected to acknowledge responsibility for any outcome in such situations (15,16).

Apology - A Definition

The structure and definition of an apology, as well as considerations to give an apology, vary according to the circumstance faced by the clinician. Yet, the four basic components of an effective apology remain the same (16,17) :

1. Acknowledgement - explaining to the patient that you are aware of the situation.
2. Explanation - sharing the facts one knows about what has happened and reporting more to the patient as it becomes known.
3. Expression of remorse and humility - showing regret about what happened and reassuring the patient to uphold the best possible standard of care, whether or not one admits or implies an admission of fault.

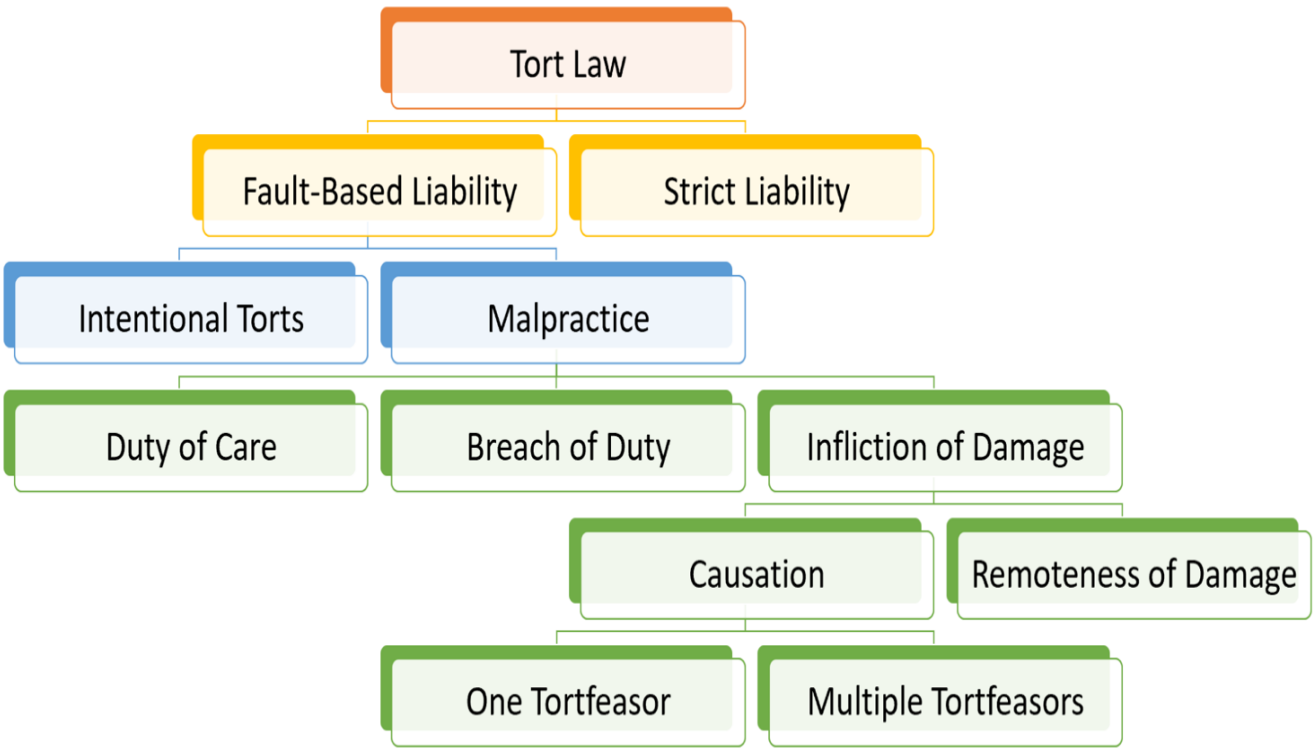

Figure 2: A Hierarchical Breakdown of Tort Law.

4. Reparation - explaining what was learnt from the outcome of the error and how one will make sure that the situation will not be repeated again.

Apology Laws and Legal Liability

The Tort System

The *tort* system is a system by which one is liable for damage which occurs through one's own fault, as explained in Figure 2 (18). This is the system currently enforced on Maltese medical practices by the Maltese Civil Code (19). While this system is important in maintaining accountability for one's actions toward another, it paves the way for potentially dangerous defensive medicine. Defensive medicine is the process of ordering additional tests or procedures primarily to avoid malpractice liability or avoiding patients or treatments out of concern for malpractice liability. Tort law implies that a physician is admitting to wrongdoing and fault by giving an apology. In this case, it is legally advised not to issue apologies to patients or their relatives in cases of medical error (20).

The No Fault System

The *no fault* system has become widely adopted in issues of liability and medical negligence. This is the case in Denmark, Finland, Sweden, Norway, Canada, New Zealand, some states in the United States of America, and some Australian provinces (7,20). The UK adopted what is considered to be legislation which is similar to the *no fault* system, as part of an act providing compensation for workers affected by asbestos exposure (21).

In this system accidents and injuries are regarded as inevitable. Proof of causation, rather than proof of fault, needs to be offered to uphold the claim. Therefore in this system, apologising for an error does not incriminate, as it may be an inevitable error. The claimant must show that the medical error was thus the causative factor in the injury sustained, irrespective of who is to blame, as explained in Figure 3 (22).

Effectiveness of Apology Laws

In a recent study, empirical evidence was used to observe the impact of apology laws with regard to settlements and lawsuits. This study concluded that for minor injuries, the law did not significantly impact the settlement payment of cases, but reduced the total number of such cases. On the other hand, cases involving significant and permanent injuries increased the number of resolved cases, while giving a decrease in the average settlement payment in these cases. The increase in the number of resolved cases is believed to be due to a reduction in the time to reach a settlement due to more protracted lawsuits. The research paper also concluded that in the long run, there could be fewer lawsuits overall (23).

In other studies, it is argued that apology laws are a form of tort law reform, and are in essence not enough. A study observing American heart attack patients concluded that apology laws do not deter defensive medicine, and increase the patient's length of stay in hospital (24). In a separate study, it was found that neurosurgeons face increased numbers of lawsuits and increased settlement payments when apology laws are in force (25).

Yet, literature suggests that while apologies themselves do make a positive impact, apology laws fail to do so. The reason behind this is that there is a lack of training of physicians and other healthcare providers in the manner and frequency that these apologies are given. Development of educational programs specific to apologies and medical error disclosure, as well as coaching and emotional support for healthcare workers, administrators, patients, and relatives promotes better use of apologies and so more effective apologies. As such, apology laws in the absence of physician and healthcare worker education are simply not enough (16,24).

Alternatives to Apology Laws

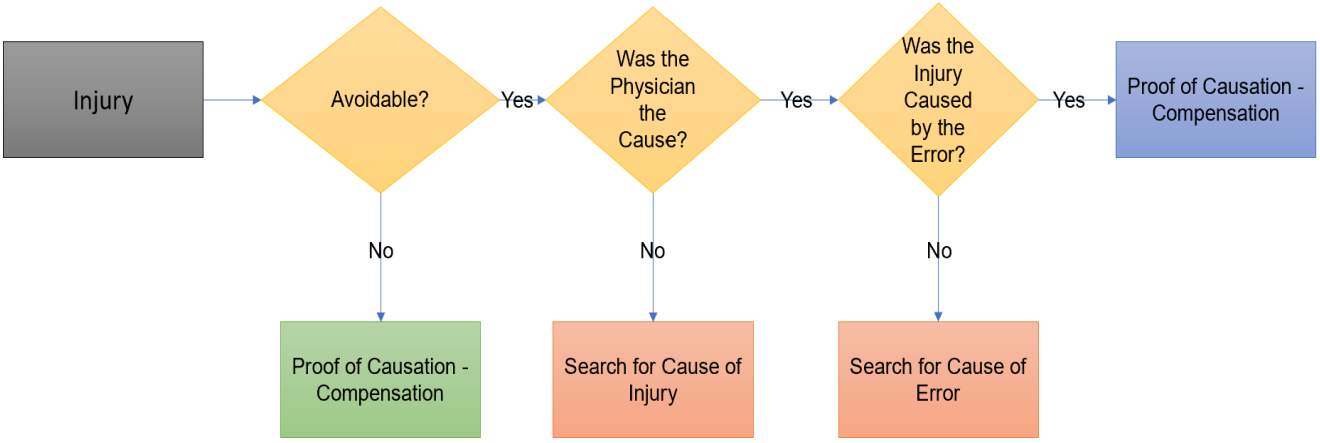

Figure 3: A Flowchart Showing the Process of Determining Proof of Causation.

Stemming from the present system, alternative dispute resolution is a method through which a claim of medical malpractice is settled between parties without involving the courts (20).

The most common practice related to this is mediation. Mediation involves the appointment of a third party, with agreement from both sides, to assist them in resolving the dispute. The mediator would then be able to identify the issues and generate and explore potential solutions. A process of mediation and communication would then be started and mediated until the two parties reach an agreement about all or part of the dispute. This would thus need the mediator to be autonomous from both parties, for the mediator to be sufficiently informed to be able to make decisions, and for the mediator to treat the dispute with absolute confidentiality. While this might be a faster and less costly option compared to litigation, the mediator does not have the authority to impose a legally-binding decision on the parties, and so the resolution of the dispute ultimately lies in the disputed parties' hands (26).

Similar to mediation, arbitration involves the use of non-judicial third parties to resolve disputes between parties. This is many times done to minimise the expenses and formality of a lawsuit. The medical system might apply this practice by requiring patients to sign a terms of service contract before undergoing certain procedures, in which the patients agree to solve any disputes which might arise out of court, in the hands of a tribunal (27). In Malta, a decision taken by a tribunal is legally binding, as long as a Notice of Arbitration is sent to the relevant authorities. Arbitration would require competency and informed consent, which some critics argue are not correctly ensured (20).

Another mechanism is pre-hearing screening. This involves the patient's lawyer submitting a request for a review of a case of malpractice to a medico-legal committee appointed jointly by a bar association and a medical association. This request would include preliminary information on the case and authorise the panel to deliberate in confidentiality on the merits of the case. The panel does not settle or compromise the claim, it only judges whether there is substantial evidence to support the claimant's allegations. The panel then delivers its judgement in a report addressed to the lawyer and the physician or physicians concerned. The deliberations and any votes taken by the panel remain secret. In cases where the panel deems that there is or might be reasonable enough evidence for professional negligence through which the claimant was harmed, it cooperates fully with the claimant to move the dispute to a court of law. In cases where the panel finds that there is no reasonable possibility of professional negligence, or harm arising from such negligence, the lawyer would refrain from filing court action unless personally certain that there are overriding reasons in the interest of the client. This system would thus weed out unreasonable claims, thus lessening the burden placed on the courts. While this is advantageous, in jurisdictions where this method is accepted, the submission of these claims is voluntary, and the decision of the panel is not binding, thus placing additional financial and time burdens on the claimant (28).

Discussions and Conclusion

It becomes apparent that there is no one perfect solution to the issue of apologies in medical practice. Yet, steps should be taken to have a universal stance on the topic, especially when considering the implications of the right to cross-border medical care within the European Union.

While there is empirical research highlighting the benefits and drawbacks of different systems through which this issue is tackled by various countries, it is many times political lobbying, rather than impartial research, which drives lawmakers to choose one over another.

Media also plays an important role in lobbying for a different legal system. In recent times, there has been an increase in media reporting and emotionalising cases, generating an infallible view of medics and the law as well as a sense of crisis. As such, both locally and internationally, there has been a recent drive to change laws, and hold physicians accountable, even when there is the shared burden of system errors.

As Alexander Pope writes in his poem *An Essay on Criticism*, "To err is human, to forgive divine." It should therefore be accepted that physicians are prone to error, as any other mortal being. Indeed, critical thinking skills and training in medical error disclosure are skills which are not emphasised in medical school but are very much needed during one's professional life in the medical sector. Consequently, these soft skills should be taught, adequately explained, and trained within medical school and also during post-graduate training (29).

Moreover, although human fallibility cannot be changed, the conditions under which one works can. Shorter hours, aid by electronic systems, and laws which protect physicians' fallibility can all be piloted and introduced to minimise medical errors. While one can try to discuss and try to eliminate errors one by one, it is more efficient to look into the actual cause and eliminate it.

In closing, apologies, when merited, result in fewer overall lawsuits, while there is conflicting evidence of the effectiveness of apology laws. When patient harm has occurred, or has the potential to occur, an apology should be offered. This is an important part of the healing process and can help to build trust between the patient and the practitioner. A doctor should discuss the complete situation with a superior or legal counsel and take into account the local jurisdiction and clinical guidelines set by their respective medical associations when they believe an apology is necessary. It is only after this consultation that a decision should be taken whether to issue an apology for each specific situation.

In situations where an apology may not be appropriate or legally advisable, there are other ways to express regret and demonstrate a commitment to addressing any issues that may have arisen. For example, practitioners can express sympathy for the patient's experience, offer to investigate the situation further and take steps to prevent similar incidents from occurring in the future.

Ultimately, the most important thing is to prioritize the patient's well-being and to take appropriate steps to address any issues that may have arisen. Whether through an apology or other means, practitioners, and students should be proactive in taking responsibility for their actions and working to ensure that patients receive the highest quality care possible.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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Genetic subtypes of Hereditary Spastic Paraplegia

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Abstract

Hereditary Spastic Paraplegias (HSPs) are a type of genetic disorders where a spastic gait is either the only or a major feature seen in affected patients. Symptoms involved include lower limb weakness and a high rate of muscle spasming. These diseases are clinically grouped into pure (uncomplicated) and complicated. Patients suffer from sensory loss in the lower limb in both types, but those with complicated HSP will experience neurological defects such as cerebellar dysfunction (tremors, ataxia, and nystagmus), ophthalmic problems such as pigmentary retinopathy and optic atrophy amongst others. HSP is categorized according to mode of inheritance (autosomal dominant, autosomal recessive, and X-linked recessive), clinical phenotype, or any neuro-pathophysiology. The vast majority of pure HSP are autosomal dominant cases, while complicated HSP cases are primarily autosomal recessive. Pathophysiology involves abnormalities in membrane trafficking and axonal transport. Treatment currently consists of stretching workouts, pharmacotherapy, and physiotherapy.

Keywords: Hereditary spastic paraplegia, genetics

Introduction

Hereditary spastic paraplegias (HSPs) are very complex genetically and clinically mixed group of patients presenting with lower limb weakness and a high rate of muscle spasming. These groups are clinically classified into: (1) uncomplicated (pure) group which is characterized with predominant pyramidal signs such as spasticity, extensor plantar responses, paraparesis, rapid tendon reflexes and deep sensory loss; and (2) complex or complicated group because the patients suffer from spastic paraplegia with further neurological disabilities. Some of these include cerebellar dysfunction (tremors, ataxia, and nystagmus); ophthalmic problems such as pigmentary retinopathy and optic atrophy; cognitive and dementia (1), (2).

Seventy-two known spastic paraplegia gene loci have been mapped and 55 associated genes discovered (3). The most common form

is autosomal dominant HSP and is found in approximately 70% of cases. The time of onset for this disease varies from childhood till 70 years of age (3). The vast majority of pure HSP are autosomal dominant cases, while complicated HSP cases are commonly autosomal recessive (2). The aim of this review article is to provide a general overview of the most common genetic subtypes of HSP.

Autosomal dominant HSP

SPAST-associated HSP or SPG4

The frequently found type of uncomplicated autosomal dominant HSP is SPAST-associated HSP, presented in 40-45% of cases (6). This type involves a problem in the SPAST gene which encodes information about the microtubule-severing protein Spastin and is the most clinically studied in the greatest amount of detail. It normally presents from childhood through late adulthood. Generally, over half of the carriers for the mutated gene will not develop symptoms before the 30 years of age. The phenotype in most cases is gradual progressive spasming in the legs with loss of movement 20 years after the onset. Symptoms in those who had the disease for a longer period consist of upper limb hyper-reflexia, urinary urgency, loss in the ability to sense vibration and muscle wasting in the lower limbs. Complicated phenotypes involve epilepsy, thinning of corpus callosum, mental retardation and cerebellar ataxia. It has been evident that progressive cognitive decline is also present in people above the age of 40 and can progress to dementia by 60-80 years (7).

SPG3A

The 2nd common cause of this type of HSP is SPG3A-associated HSP, presenting in 10% of case. It is also the most common cause of HSP appearing before 10 years of age (5). ATL1 encodes for Atlastin 1 and mutations of this protein are mainly of the missense type as well as in-frame and whole genome deletions (5,7). Onset typically appears around age 4 and solemnly, later than that. It generally has an uncomplicated phenotype but there may be further symptoms such as a thin Corpus Callosumand axonal polyneuropathy of small hand muscles (Silver syndrome). Severe spastic gait is found in patients with very early onset unlike in later onset (5,7).

SPG6

Mutations in SPG6 also cause this type of HSP but symptoms can become severe associated with rapid spasticity, polyneuropathy, epilepsy and cognitive impairment. The degree of penetrance is very high and depends on age. SPG6 can be brought about by mutations in NIPA1 (Non-Imprinted in Prader-Willi/Angelman syndrome region protein 1). (7,9).

SPG8

SPG8 is responsible for the protein Strumpellin (10) which is commonly expressed and found in endoplasmic reticulum and cytoplasm. Clinical manifestation is pure type HSP and mutations in the SPG8 are described to have severe spasms and low vibration sense. There is also a reduction in axonal outgrowth with loss of function that interrupts the endosome membrane trafficking (7)

SPG10

This disease is due to mutations in KIF5A gene which has the neuronal kinesin heavy chain encoded in it. This chain is important for anterograde axonal transport. It usually presents as pure SPG along a late onset of the phenotype (1).

SPG12

SPG12 is caused by mutated RTN2 gene that encodes the protein reticulon 2. The latter is involved in ER shaping and interacts with Spastin. Presentation is pure rapidly progressive spastic paraplegia, and the onset is early on in life (1).

SPG13

Mutations in the HSP60 gene will give a late onset without any complications. Some patients carrying the SPAST mutations have been found to have a Gly563Ala missense variant without any pathogenic changes. But a mutation in Asp29Gly causes early onset neurodegenerative Pelizaeus-Merzbacher-like disease only when the alleles are in homozygous state (7).

SPG17

SPG17 mutations cause a problem in Seipin coded by the BSCL2 gene (1), an ER integral membrane protein with an unknown function. It presents as a complicated form that is

Table 1: Identified HSP genes, grouped by mode of inheritance (1,4,5).

	Disease	Gene	OMIM Gene/locus	Protein name	Functional Modules	Cellular Functions
Autosomal Dominant	SPG4	SPAST	MIM604277	Spastin (M1 and M87 isoforms)	Membrane traffic and organelle shaping	ER morphogenesis Endosomal traffic BMP signaling Cytokinesis
	SPG3A	ATL1	MIM606439	Atlastin-1	Membrane traffic and organelle shaping	ER morphogenesis Bone Morphogenetic Protien signaling
	SPG6	NIPA1	MIM608145	NIPA1	Membrane traffic and organelle shaping	Endosomal traffic Mg2+ transport BMP signaling
	SPG8	KIAA0196	MIM610657	Strumpellin	Membrane traffic and organelle shaping	Endosomal traffic Cytoskeletal (actin) regulation
	SPG10	KIF5A	MIM602821	KIF5A	Membrane traffic and organelle shaping	Microtubule-based motor protein
	SPG12	RTN2	MIM603183	Reticulon 2	Membrane traffic and organelle shaping	ER morphogenesis
	SPG13	HSPD1	MIM118190	HSP60 chaperone	Mitochondrial regulation	Mitochondrial chaperonin
	SPG17	BSCL2	MIM606158	Seipin	Membrane traffic and organelle shaping	Lipid droplet biogenesis at ER
	SPG31	REEP1	MIM609139	REEP1	Membrane traffic and organelle shaping	ER morphogenesis ER-microtubule interaction

	SPG33	ZFYVE27	MIM610243	Protrudin	Membrane traffic and organelle shaping	Involved in the intracellular trafficking
	SPG42	SLC33A1	MIM603690	Acetyl-CoA transporter	Myelination and lipid/sterol modification	Acetyl-CoA transporter
Autosomal recessive	SPG5A	CYP7B1	MIM603711	OAH1	Myelination and lipid/sterol modification	Cholesterol metabolism
	SPG7	SPG7	MIM602783	Paraplegin	Mitochondrial regulation	Mitochondrial m-ATPase associated with numerous cellular activities ATPase
	SPG11	KIAA1840	MIM610844	Spatacsin	Membrane traffic and organelle shaping	Endosomal traffic
	SPG15	ZFYVE26	MIM612012	Spastizin	Membrane traffic and organelle shaping	Endosomal traffic Cytokinesis Autophagy
	SPG18	ERLIN2	MIM611605	SPFH2	Membrane traffic and organelle shaping	ER-associated degradation Lipid raft-associated
	SPG20	SPTG20	MIM607111	Spartin	Membrane traffic and organelle shaping	Endosomal traffic BMP signaling Cytokinesis Lipid droplet turnover Mitochondrial regulation
	SPG21	ACP33	MIM608181	Maspardin	Membrane traffic and organelle shaping	Endosomal traffic
	SPG35	FA2H	MIM611026	Fatty acid 2-hydroxylase	Myelination and lipid/sterol modification	Myelin lipid hydroxylation
	SPG39	PNPLA2	MIM603197	Neuropathy target esterase	Myelination and lipid/sterol modification	Phospholipid homeostasis
	SPG44	GJC2	MIM603197	Connexin-47	Myelination and lipid/sterol modification	Intercellular gap junction channel
	SPG48	KIAA0415	MIM613653	KIAA0415	Membrane traffic and organelle shaping	Endocytic adaptor protein complex
	AP-4 deficiency	AP4B1 AP4M1 AP4E1 AP4S1	MIM607245 MIM602296 MIM607244 MIM607243	AP-4 S1 B1, and E1 subunits	Membrane traffic and organelle shaping	Endocytic adaptor protein complex
X-linked	SPG1	L1CAM	MIM308840	Neural cell adhesion molecule	Myelination and lipid/sterol modification	Cell adhesion and signaling
	SPG2	PLP1	MIM300401	Proteolipid protein 1	Axon Pathfinding	Major myelin protein

portrayed by having extra amyotrophy of small muscles in hands and feet with symptoms showing up in adolescence to late 30s. This is known as Silver syndrome and it may also trigger hereditary motor neuropathy type V, Charcot Marie Tooth disease 2 and Berardinelli-Seip congenital lipodystrophy which is an autosomal recessive disorder (7).

SPG31

Mutations in Receptor Accessory Protein 1 contribute to the pure form of HSP (4) where onset can occur at any age. 31 different mutations have been observed in 37 families. 16 of which actually show a pure HSP phenotype. There were reports of 10 different heterozygous point mutations and 2 intragenic exon deletions. This disease can also manifest with Silver syndrome (1,7,11).

SPG33

SPG33 base change was originally thought to bring about pure HSP. This protein is associated with Spastin and maybe considered important for endosomal transport. The heterozygous mutation occurs in ZFYVE27 on chromosome 10q24 which encodes a Spastin binding protein protrudin, which is a member of the FYVE-finger family of proteins(12). Mutation in this protein causes an impairment in its interaction within Spastin and can affect neuronal intracellular trafficking in the corticospinal tract (5,7).

SPG42

The gene involved is SLC33A1, which is responsible for Acetyl-CoA transporter. Onset is between 4 and 42 years (1).

Autosomal recessive HSP

SPG5A

Patients with autosomal recessive HSP have mutations in the SPG5A gene. This is an uncomplicated form of HSP where onset occurs at any age and the progression of the disease slowly builds up. SPG5A is brought about by a mutation in the CYP7B1 gene encoding for oxysterol-7α-hydroxylase. This substance is responsible for the cholesterol degradation into bile acids. This type of HSP can be detected by elevated levels of 27-hydroxycholesterol and 25-hydroxycholesterol in the CSF and plasma (7).

SPG7

This involves the SPG7 gene which encodes for Paraplegin and mutations of the latter are responsible for 5% of autosomal recessive cases. Age of onset is around 30 years whereby patients present with ataxia and progressive spasticity (3). This type of mutation causes the pure and complicated phenotypes with common conditions for the latter consisting of pale optic discs, peripheral neuropathy, ophthalmoplegia (3) and cerebral signs (nystagmus ataxia and dysarthria) (7).

SPG11

SPG11 mutations occur on the SPG11 locus on chromosome 15. Onset is in adolescence (mean age is 14.3 years) whereby the patient develops problems in walking, ataxia, complications in the bladder, spasticity, possessing a thin corpus callosum, parkinsonism, cognitive impairment, and severe neuropathy. In a case report, a patient presented with very mild symptoms such as brisk reflexes, walking on toes and extension of the foot at 12 years of age. This patient had little progression of the disease after 10 years of onset. The aunt of this patient developed

the symptoms at the age of 30. Another case had spastic paraplegia with visual problems and very severe optic atrophy (7).

MRI findings include signs of metabolic and structural tissue damage in the brain. There have been features in cases such as diffuse brain volume decrease mostly in the Neocortex and less so in the White Matter and decrease as well in N-acetyl aspartate/Creatinine Ratio which indicates dysfunction(13).

SPG15

The involved gene in this paraplegia is ZFYVE26. Encoded in this gene is information about a zinc-finger protein with a FYVE domain, named Spastizin. The latter interacts with Spatacsin (found in SPG11) and KIAA0415 (SPG48). It presents clinically with dysarthria, mild cerebellar signs, spastic quadriparesis, parkinsonism and intellectual disability (1).

SPG18

This is due to a nullimorphic deletion of ERLIN2 gene, responsible for a section of the ER associated degradation system for proteins. Presentation includes progressive weakness, intellectual disability and spasticity (1).

SPG20 and SPG21

SPG20 contains information regarding the protein Spartin and a mutation in this gene causes Troyer syndrome. SPG21 encodes for Maspardin and a mutation in this gene causes Mast syndrome. Troyer syndrome causes spastic tetraparesis, distal amyotrophy, short stature, dysarthria, and a difficulty in learning. Contrastingly, patients with Mast syndrome present with cerebellar and extrapyramidal signs, thin corpus callosum and dementia. Both of these mutations cause complicated HSP, and both are due to a founder mutation in the Old Order Amish population (5).

SPG35

SPG35 codes for the FA2H enzyme which is responsible for the synthesis of sphingolipids possessing 2 hydroxy fatty acids which are important for many biological processes. Studies in mice have revealed that this protein is essential for the long-term maintenance of myelin. Mutation of SPG35 is characterized by cognitive impairment, leukodystrophy on MRI, gait difficulties and dysarthria. This mutation is involved in another two neuropathophysiological diseases: neurodegeneration with accumulation of iron in the brain and leukodystrophy along with dystonia and spastic paraparesis (5).

SPG39

Neuropathy target esterase gene mutations is the cause for this disease. The latter encodes phospholipase B/lysophospholipase. Patients suffering from this disease experience the pure phenotype and there is associated waisting of distal leg and intrinsic hand muscles (1).

SPG44

The mutated gene is GJA12/GJC2, which encodes the gap junction protein connexin-47. This paraplegia has a late onset and presents slowly and progressively. Hypomyelination can be noted on MR spectroscopy (1).

SPG48

The gene affected is KIAA0415. This holds information regarding putative helicase, a protein having a role in DNA breaks by a process called homologous recombination DNA double-strand break repair (HR-DSBR). This protein also interacts with spastizin (SPG 15) and spatacsin (SPG11), Presents with pure phenotype with urinary incontinence and age of onset is 50 years (1).

AP-4 deficiency

This is a collection of neurodegenerative disorders denoted by a progressive, complex spastic paraplegia. Onset of AP-4 deficiency is in infancy or early childhood. Patients present with neonatal hypotonia of all the limbs with complications such as dysphagia, foot deformities, contractures and dysregulation of the bowel and bladder function. Severe intellectual disability is also seen in these patients. Diagnosis is possible by identification of mutations in genes encoding heterotetrametric adaptor protein 4 complex (AP4) subunits: SPG47/AP4B1, SPG51/AP4E1, SPG52/AP4S1 and SPG50/AP4M1 (5,14).

X-linked HSP

SPG1

This causes mental retardation, adducted thumbs, hydrocephalus, and spasticity of lower limbs. The phenotype of this disease involves X linked hydrocephalus and failure of development of corpus callosum MASA syndrome which stands for Mental retardation, aphasia, spastic paraplegia, and adducted thumbs. The X linked hydrocephalus is coupled with stenosis of the aqueduct of Sylvius (5).

SPG2

SPG2 is another gene which can cause X linked HSP. This gene codes for Proteolipid Protein and mutations at the Xq21-q22 (Chromosome X long arm, band 2 sub band 1- long arm band 2 sub band 2) contribute mostly to the complicated form which is correlated with white matter changes visible on MRI and peripheral neuropathy.

The other rare type of X linked HSP is SPG16 which involves motor aphasia, reduced vision, sphincter disturbance, quadriplegia, and mild mental retardation (5).

Non-SPG HSP

Even though there are currently 50 different types of SPG mutations identified, the genetic heterogeneity of SPG syndromes themselves can surpass this amount. Due to the phenotypes being so variable in multiple neurological diseases, as well as rare and atypical phenotypes happening, there is a connection between HSP and other neurodegenerative diseases.

In the case that the patient develops ataxia, autosomal recessive ones and spinocerebellar ataxias need to be listed as differential diagnoses. One type of ataxia termed Friedreich's ataxia has the possibility to become spastic paraplegia in adulthood with gait ataxia as well.

The presence of a dysphagia and pseudobulbar dysarthria or involvement of the upper limb can indicate motor neuron diseases like primary lateral sclerosis and Amyotrophic lateral sclerosis (ALS). If peripheral nerves are involved, hereditary neuropathies should be considered as well (15).

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

Conclusion

HSP is considered as a genotypically and phenotypically diverse set of monogenic diseases which has new possibilities to offer precision medicine and specific molecular therapies. The increased research in genes has identified many potential mechanisms for axonal degeneration (16). Being able to locate more causative genes aids in development of functional routes taking part in these diseases with the aim of targeting these routes with common drugs (1). Current treatment currently consists of reducing muscle spasticity by stretching workouts and pharmacotherapy like lioresal, tizanidine or dantrolene (1,3,17). Physiotherapy is essential to improve leg strength and gait, focusing first on stretching then balance. Stretching for a certain amount of time will also enhance the cardiovascular system. Prognosis of disease is limited to how severe the disease manifests and the age of onset. It is generally improved by administrating medications for spasticity (17).

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Disorders of the Olfactory & Gustatory Systems and their role in COVID-19, Parkinson's & Alzheimer's Disease

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Abstract

The chemical senses of the body are more extensive than one may think. Although research has progressed on both the olfactory and especially gustatory systems, there is still a constant race to determine any new findings possible related to these systems. Even though these are two separate systems, they are dependent on each other. This review describes various disorders associated with both smell eg: anosmia and hyperosmia; and taste for example ageusia, and parageusia. The correlation between COVID-19, Alzheimer's & Parkinson's Disease with olfactory and gustatory disorders is also discussed.

Keywords: Olfactory system, gustatory system, COVID-19, Parkinson's and Alzheimer's Disease

Introduction

Olfactory disorders

Upon increasing age, the risks for olfactory disorders increase. Like in taste, all olfactory disorders are split into two categories: qualitative and quantitative disorders (1).

The disorders however can also be divided into their different points of origin (2), which are:

i. Conductive: which is peripheral
ii. Sensorineural: which is of central origin

Conductive disorders are caused by the inability of odorants to make their way to the olfactory epithelium and Olfactory

Receptors (refer to Figure 1). This is due to an anatomical barrier. Central disorders are caused due to a lack of processing of information by the olfactory receptors (OR), olfactory receptor neurons (ORN) or even by more central areas like the Central Nervous System as well as any central pathways leading to it (2).

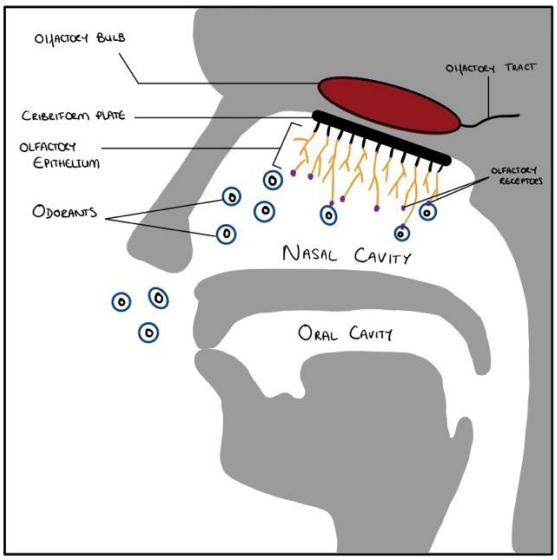

Figure 1: Diagram illustrating the Olfactory System in the human body and the initial phase of olfaction.

Quantitative Disorders

These two disorders are classified as Quantitative Disorders as they directly impact the amount a person smells and not the type of smell (1).

Anosmia is an olfactory disorder where there is a complete loss of smell. Hyposmia refers to a disorder where a person's smell decreases drastically but is still somewhat present although at a very suppressed level. Both these disorders can have quite a huge impact on a person's way of life as well as their health (3).

Many studies throughout the years have discussed and evaluated the relationship between anosmia and hyperosmia with eating disorders resulting in excessive weight loss. It is also a massive disadvantage to lose your sense of smell with respect to health risks. It can result in a person consuming food that is spoiled, rotten or even just consuming something which is not meant to be eaten (4). This can impose certain implications on the persons health such as gastrointestinal and digestive issues.

Hyperosmia is the least common of all quantitative disorders. This is when a person's ability to smell is intensified to the extreme and thus they smell excessively, contrary to hyposmia. In a past study, a link between hyperosmia caused by Lyme Disease was discovered (5).

Qualitative Disorders

These types of disorders affect the quality of smell i.e., they inhibit a person from correctly smelling something and being able to identify a particular odorant (1). The disorders are Parosmia and Phantosmia.

In Parosmia, a person has a dysfunction in the specificities of smell detection. The brain begins to identify it as an unpleasant smell (such as: rotting, burning, faecal or chemical smell), hence the odour is distorted (1). As of yet, there is still no cure for parosmia but there are medications that can help with the degree of severity of parosmia (6).

Parosmia can be divided into peripheral and central types but can also be a combination of both in certain cases (7). Some examples of central parosmia's are as follows:

i. When pregnant women tend to have particular illusions on certain smells.
ii. Hallucinations of odours (phantosmia) may occur in mental illnesses such as schizophrenia (7).

Examples of Peripheral Parosmia's include:

i. Cacosmia is when a patient is constantly smelling bad odours. This can be due to epilepsy (6).
ii. Essential parosmia is when a person continues to carry out the function of olfaction without any proper olfactory impression and like in cacosmia results in a distasteful odour (6).

Phantosmia is another type of hallucinative disorder where a person is convinced, they are smelling a particular odour without even taking a sniff of air. This usually is always caused by a tumour (6).

Taste Disorders

Taste disorders have quite adverse effects on one's overall health as well as their way of life. These types of disorders may lead to an even worse situation that can result in an eating disorder such as anorexia, which is caused by malnutrition (8). Taste disorders may also cause mental battles such as depression since the food tends to bring an element of joy to people's livelihoods thus when the element of taste is removed, as is the happiness that comes along with it.

Although it is widely known that loss of taste has such an impact on patients' lives, there is yet still no medication or any sort of treatment which can improve this dysfunction (8). The taste-related disorders we will be discussing are Ageusia, Hypogeusia, Parageusia (dysgeusia) and Gustatory Hallucinations (Table 1).

Quantitative Disorders

Starting with Ageusia, which is when there is a complete loss of taste (9). Hypogeusia is when there is a partial loss of taste function resulting in a decreased overall ability to taste (10). As well as Hypergeusia, which is the exact opposite of hypogeusia. thus, instead of barely smelling anything at all, this disorder causes excessive smelling. This is due to elevated responsiveness to all types of tastants (9). These disorders are collectively referred to as Quantitative Disorders since they all influence directly the amount of taste perception and not the type of taste perception.

Qualitative Disorders

These types of disorders are called Qualitative as they directly impact the quality as well as the type of taste which is being perceived (10). Firstly, Parageusia is a disorder whereby a person has a very noticeable taste distortion and usually this results in them tasting certain foods that they used to enjoy before, as completely horrible now (10). This may also cause them to fixate more on certain foods which prior to this disorder they would not usually tend to go for due to the taste. Some people with parageusia have also noted a more prominent metallic taste in certain foods they eat.

The next disorder is phantogeusia which is when specifically, one starts to hallucinate certain tastes which realistically are not present. So for example: upon eating

something sour, the person with phantogeusia is convinced that they are tasting something sweet when in reality they are tasting the sour food but are just not mentally making the connection from mouth to the brain. This concept may be hard to understand as it is quite different from the other disorders as this stems mainly from a mental point of view and not as much from the taste bud aspect. It can also be defined as when somebody is tasting a specific taste which is happening during the absence of a tastant (9).

Dysgeusia is a more collective term that refers to both parageusia and phantogeusia (10).

Clinical Taste Disorders (primary symptom)	Causes
Ageusia (complete loss of taste)	• Nerve Lesion to the Lingual nerve & CN IX • Hypothyroidism • Crohn's Disease • Pernicious Anaemia • Excessive inflammation of the Olfactory Pathway
Hypogeusia (A greatly decreased ability to taste)	• Drug use • Zinc Deficiency
Parageusia (Distortion of taste)	• Medications • Chemotherapy • Pregnancy • Zinc Deficiency
Phantogeusia (Gustatory hallucinations)	• In the absence of food or drink • Occurs after taste loss due to viral Infection

Table 1: The relation of Taste Disorders to their causes. Table comparing the different types of taste disorders with their respective symptoms and causes. Ageusia is the rarest of all disorders. Adapted from Purves *et al.,* 2011.

Discussion

Effects of Inflammation and Viral Infections on Taste Function

Inflammation continues to be a major contributing element in all diseases associated with taste disorders. Inflammation which is caused by certain viral infections that tend to attack the upper region of the airway tract i.e. the larynx, as well as the upper region of the gastrointestinal tract such as the mouth and pharynx are heavily associated with the causes for taste dysfunction (8). Hepatitis B is another type of infection that specifically affects the gustatory system by increasing its sensitivity to different tastants. There are also diseases such as Lupus which is characterised as an auto-immune disease that also affects one's ability to taste (8).

Toll-like receptors (TLRs) secrete chemicals such as cytokines or interferons. These are secreted due to an inflammatory response that was stimulated by a foreign body i.e. a pathogen. Upon it entering the body, the pathogen is engulfed by cells that form part of our immune system. These are known as antigen-presenting cells, as they present an antigen on their cell surface which will in turn activate the TLR (8). Although the connection between TLR activation and taste dysfunction has been established, there are still many more discoveries to be done in order for this great suspicion to become concrete facts.

The Effects Of COVID-19, Parkinson's & Alzheimer's on taste and smell perception

COVID-19

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the virus that causes COVID-19, the coronavirus. A trend was noticed in patients who contracted this illness which has caused a worldwide pandemic. This trend was such of Olfactory and gustatory disorders. Some people reported symptoms such as anosmia only, and some complained of ageusia only however most of the people reported to have experienced both loss of taste as well as loss of smell (12)

Why is taste and smell lost? Although not much extensive research has been done in the past on why the sense of taste is lost, one thing is certain, which is that for the most part, our ability to taste is stemming from our capability of smelling different odours. Thus, the main pathological issue created by COVID-19 is more specific to the olfactory epithelium rather than solely to the taste buds in the mouth (13).

The mucus found in the nasal region is essential to trap odorant molecules which are then detected via the olfactory receptor neurons. In the case of COVID-19, once these viral cells enter the nasal cavity, they begin to interact with the ACE2 receptors found on the target cells within the mucous layer (14). This does not occur only in the nose but also in the mucous membrane in the oral cavity, which in this case is specifically found on the tongue. Thus, in both cases, SARS-CoV-2 uses the Angiotensin-converting enzyme 2 (ACE2) receptor to make its way into cells by binding to ACE2 using its spike proteins (12). Some researchers have also discovered that apart from SARS-CoV-2 requiring the ACE2 receptors on certain cells, it also needs the protease, TMPRSS2 which aids in the binding of the spike protein, which is found on the COVID-19 cell surface, to the ACE2 receptor (13). Both the ACE2 receptor and the TMPRSS2 are found also in the throat and upper respiratory throat apart from the nose and mouth which further explains why apart from ageusia, and anosmia infected people also present with sore throats, severe coughing and shortness of breath.

Since this virus has only recently begun to transcend in the world of infectious diseases, more research is still yet to be done to justify any further relations between specific neurological pathways which may also be causing such olfactory and gustatory disorders.

Parkinson's & Alzheimer's Disease

Parkinson's Disease and Alzheimer's Disease are both neurodegenerative diseases. Parkinson's disease affects the dopaminergic neurons of the brain and affects problem-solving, speed of thinking, memory and mood (14). Whilst Alzheimer's disease is when there is atrophy of the brain and hence affects language, memory and thinking skills (15).

In Parkinson's Disease, the majority of the pathophysiology is occurring within the olfactory bulb as this is the final stage where the processing of olfactory information occurs, in fact, it is referred to as the 'Olfactory Thalamus' (16). In Parkinson's patients, only a few tend to report complete anosmia whilst many patients complain about hyposmia (thus a greatly reduced smelling function). Also, the ability for one to be able to differentiate between different odours as well as recognise an odour is

severely impacted in people with Parkinson's Disease. Severe loss of smell is also an early indication of Parkinson's disease thus it is generally used as a biomarker to diagnose the patient with Parkinson's. The exact cause of dysfunctional smell in Parkinson's Disease is still unknown but is thought to be caused by the changes in dopamine (a neurotransmitter) levels in the brain caused by this neurodegenerative disease (16 – 18).

Alzheimer's Disease is one of the most common diseases in the elderly when it comes specifically to neurodegenerative diseases. An identifiable marker of Alzheimer's is dementia. The first part of the brain which is impacted by this neurodegenerative disease is the olfactory system and hence this is also a biomarker for the diagnosis of Alzheimer's like in Parkinson's Disease as this symptom is presented very early on in the disease (15, 17).

Conclusion

From the cumulative information collected in the above literature review, it can be concluded that both sensations of smell and taste are vastly interlinked. This leads to certain issues when one of the systems starts to malfunction as due to the extensive and cohesiveness of the functionality of both systems, the other system will become dysfunctional too. Saying this, when discussing specific illnesses such as COVID-19, one can truly understand why when infected, smell and taste most of the time are lost simultaneously. This review also allows us to appreciate the importance of Olfaction and Gustation when diagnosing or noticing even more severe diseases such as Alzheimer's and Parkinson's. Their dysfunction acts as biomarkers which are clear indications of these diseases. No matter how often overlooked, the Olfactory system and the Gustatory system have been shown to be essential functions in one's day-to-day life, which must be cared for and looked after because, unlike other disorders, most of the time, when taste or smell is lost, it proves to be very hard to regain that function. Medications aimed at improving taste or smell dysfunction are little to none and sometimes can also worsen the situation.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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Delving into the ICD-11: A Review of the Chapter on Mental, Behavioural or Neurodevelopmental Disorders

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Abstract

The International Classification of Diseases and Related Health Problems (ICD) for mortality and morbidity statistics forms the basis of global health trends and statistics identification. The mental, behavioural and neurodevelopmental (MBND) chapter of the eleventh edition of the ICD (ICD-11) was the largest and most participative process throughout the history of mental health disorder classification. This review aims to provide a comprehensive overview of the newly added disorders in ICD-11 in comparison to the tenth edition of the ICD (ICD-10) and to explain the changes and implications of approach and structure. Google Scholar and PubMed databases were searched for articles from 1899 to 2022 using search terms 'ICD-10' or 'ICD-11' and 'differences' or 'newly added disorders' or 'changes'. Only articles on the varying aspects of the ICD-11 from the ICD-10 with respect to approach, structure and new disorders were included. 53 articles were eligible for inclusion. Literature reported that the ICD-11 takes a developmental approach to classifying disorders. As a result, sleep-wake and sexual health-related disorders were cross-listed to new chapters accordingly and new disorders were added. Namely, catatonia, bipolar type II disorder, body dysmorphic disorder, olfactory reference disorder, hoarding disorder, excoriation disorder, complex post-traumatic stress disorder, prolonged grief disorder, binge eating disorder, avoidant/restrictive food intake disorder, body integrity dysphoria, gaming disorder, compulsive sexual behaviour disorder, intermittent explosive disorder and premenstrual dysphoric disorder. Limitations were the inclusion of older articles to explain newly added disorders when recent relevant ones could not be identified. Evidently, the new additions to the MBND chapter of ICD-11 have proved to be the first major revision of the world's foremost mental disorder classification in nearly 30 years. The new disorders along with other significant changes in the pre-existing mental disorders, have facilitated the clinical approach and management of psychiatric disorders by psychiatrists and non-specialists alike.

Keywords: ICD-11, ICD-10, behavioural disorders, mental disorders, neurodevelopmental disorders

Introduction

The International Classification of Diseases and Related Health Problems (ICD) for mortality and morbidity statistics forms the basis of global health trends and statistics identification (1). The pre-final version of the eleventh edition of the ICD (ICD-11) was released to the World Health Organisation (WHO) in June 2018 which was then reviewed and amended by 194 member states (2). This was approved by the World Health Assembly and the transition to utilising ICD-11 occurred (3). Notably, four ICD-11 chapters were coordinated by the WHO Department of Mental Health and Substance Abuse. These chapters include mental, behavioural and neurodevelopmental disorders (MBND); sleep-wake disorders; diseases of the nervous system; and conditions related to sexual health (4).

Interestingly, the MBND chapter of ICD-11 was the largest and most participative process throughout the history of mental health disorder classification (5). In fact, the WHO Department of Mental Health and Substance Abuse appointed the International Advisory Group for the revision of ICD-10 Mental and Behavioural Disorders in 2007 (6). Eight years later, the ICD-11 MBND beta draft was made publicly available online for review and comments (7). Formative field studies provided additional review from mental health practitioners to finally, formulate the ICD-11 that was made available online on the eleventh of February 2022 (8–10).

This review aims to provide a comprehensive overview of the newly added disorders in ICD-11 in comparison to the tenth edition of the ICD (ICD-10). It aims to emphasise on the sixth chapter in ICD-11 entitled "Mental, Behavioural or Neurodevelopmental Disorders" especially the newly added disorders. The review also aims to explain the changes in approach and structure in the sixth chapter of the ICD-11 and its implications.

New Mental, Behavioural and Neurodevelopmental Disorders in ICD-11

In comparison to ICD-10 MBND chapter, which contains 11 disorder groupings, the ICD-11 MBND chapter contains 21 disorder groupings (11). An overview of such groupings in both versions of ICD is shown in figure 1. As also depicted by figure 1, the structure greatly varies aswell since, ICD-10 was based on Kraepelin's Textbook of Psychiatry and ICD-11 followed a developmental perspective (4,12). Notably, in ICD-11 sleep-wake and sexual health-related disorders were separated from the MBND chapter and cross-listed to the new chapters related to sleep-wake disorders and conditions related to sexual health. Importantly, ICD-10 gender identity disorders were renamed as "gender incongruence" in ICD-11 and moved to the new sexual health chapter. The removal of gender incongruence from the MBND chapter emphasises how transgender identities are now not considered a mental disorder (13).

The MBND chapter in ICD-11 was based on three significant pillars namely, global applicability, scientific validity and clinical utility (4,11,17). Clinical utility is a widely

used term to describe the usefulness, practicality, and relevance of a clinical tool, in this case, ICD-11 (18). Global applicability was an approach that the WHO embraced by establishing a Global Clinical Practice Network (GCPN) to allow mental and primary healthcare professionals from all over the world to participate in the compilation of ICD-11 (4). In fact, Arab studies concluded that psychiatric classifications of disorders were improved, thus improving the ICD-11's clinical utility across all cultures. The same study emphasised how vital this is since mood, anxiety and stress-related disorders were reported as the most common psychiatric manifestations impacted by cultural factors (19). Scientific validity remains a vital standpoint of every publication to date thus, changes to existing psychiatric disorders and addition of newer ones ensure further improvements to clinical utility and global applicability (4).

Based on a review of the available ICD-10 and ICD-11 MBND chapters, a number of new disorders have been identified in ICD-11. A description of these disorders as defined in ICD-11 diagnostic guidelines and the potential rationale for their inclusion are described in the following chapters.

Catatonia

Catatonia is a psychomotor disorder characterised by the co-occurrence of several symptoms effecting psychomotor activity. All diagnostic requirements apply to all categories of catatonia namely, catatonia associated with another mental disorder; medication/substance-induced catatonia; and

Table 1: Diagnostic groupings in Kraepelin's Textbook of Psychiatry, ICD-10 and ICD-11. Adapted from Kraepelin *et al.*, 1912, ICD-10, 2016, Gaebel *et al.*, 2017, ICD-11, 2022.

Kraepelin's Textbook of Psychiatry		**ICD-10**		**ICD-11**	
Chapter Number	***Chapter Name***	***Chapter Number***	***Chapter Name***	***Chapter Number***	***Chapter Name***
I	Infection Psychoses	F00-F09	Organic, including symptomatic, mental disorders	6D70-6E0Z	Neurocognitive Disorders
				8A20-8A2Z	Disorders with neurocognitive impairment
II	Exhaustion Psychoses	F43.0	Acute Stress Reaction	QE84	Acute Stress Reaction
III	Intoxication Psychoses	F10-F19	Mental and behavioural disorders due to psychoactive substance use	6C40-6C5Z	Disorders due to substance use or addictive behaviors
IV	Thyroigenous Psychoses	F02.8	Dementia in other specified diseases classified elsewhere	6D85.Y	Dementia due to other specified diseases classified elsewhere
V	Dementia Praecox	F20-F29		6A20-6A2Z	Schizophrenia or other primary psychotic disorders

			Schizophrenia, schizotypal and delusional disorders	6A40-6A4Z	Catatonia
VI	Dementia Paralytica	F02.8	Dementia in other specified diseases classified elsewhere	6D85.Y	Dementia due to other specified diseases classified elsewhere
VII	Organic Dementias	F00-F09	Organic, including symptomatic, mental disorders	6D70-6E0Z	Neurocognitive Disorders
				8A20-8A2Z	Disorders with neurocognitive impairment
VIII	Involution Psychoses	F20-F29	Schizophrenia, schizotypal and delusional disorders	6A20-6A2Z	Schizophrenia or other primary psychotic disorders
IX	Manic Depressive Insanity	F30-F39	Mood [affective] disorders	6A60-6A8Z	Mood disorders
X	Paranoia	F22.0	Delusional Disorder	6C40.6Z	Alcohol-induced psychotic disorder, unspecified
		F10.5	Alcoholic Paranoia		
		F03	Unspecified dementia		
XI	Epileptic Insanity	G40.2	Localization-related (focal)(partial) symptomatic epilepsy and epileptic syndromes with complex partial seizures	8A	Epilepsy or seizures
XII	The Psychogenic Neuroses	F40-F48	Neurotic,stress-related and somatoform disorders	6B00-6B0Z	Anxiety or fear-related disorders
				6B20-6B2Z	Obsessive-compulsive or related disorders
				6B40-6B4Z	Disorders specifically associated with sress
				6B60-6B6Z	Dissociative disorders
				6C20-6C2Z	Disorders of bodily distress or bodily experience
/	/	F50-F59	Behavioural syndromes associated with physiological disturbances and physical factors	6B80-6B8Z	Feeding or eating disorders
				6E20-6E2Z	Mental or Behavioural disorders associated with pregnancy, childbirth, or the peurperium

				6E40-6E40Z	Psychological or Behavioural factors affecting disorders or diseases classified elsewhere
XIII	Constitutional Psychopathic States (Insanity of Degeneracy)	F60-F69	Disorders of adult personality and behaviour	6C70-6C7Z	Impulse control disorders
XIV	Psychopathic Personalities	/	/	6D10-6D11.5	Personality disorders and related traits
				6D30-6D3Z	Paraphilic disorders
				6D50-6D5Z	Factitious disorders
				7A00-7A0Z	Insomnia disorders
				7A20-7A2Z	Hypersomnolences disorders
				7A60-7A6Z	Circadian rhythm sleep-wake disorders
				HA60-HA6Z	Gender incongruence
XV	Defective Mental Development	F70-F79	Mental Retardation	6A00-6A00.Z	Disorders of intellectual development
		F80-F89	Disorders of psychological development	6A00-6A06.Z	Neurodevelopmental disorders
/	/	F90-F98	Behavioural and emotional disorders with onset usually occurring in childhood and adolescence	6C00-6C0Z	Elimination disorders
				6C90-6C9Z	Disruptive behavioural or dissocial disorders
/	/	F99	Unspecified mental disorder	6E60-6E6Z	Secondary mental or Behavioural syndromes associated with disorders or diseases classified elsewhere

secondary catatonia syndrome. Catatonia is commonly associated with schizophrenia, other primary psychotic disorders, mood disorders and neurodevelopmental disorders especially autism spectrum disorder (ASD). Substance-induced catatonia often occurs during or soon after intoxication or withdrawal of psychoactive substances. These include phencyclidine (PCP) and cannabis, among others. Conversely, secondary catatonia is secondary to another medical illness not classified in the MBND chapter namely, hypercalcemia, diabetic ketoacidosis or hepatic encephalopathy (16).

As shown in figure 2, symptoms vary depending on the effect on psychomotor activity. Essentially, diagnosis must include three or more of such symptoms that can be coming from one symptom cluster or a combination of the three shown clusters. Symptoms usually last for at least several hours but can persist for longer. As a result, impairing daily functioning or causing medical complications such as contractures, dehydration or aspiration (16).

In ICD-10, catatonic symptoms were included in the definitions of catatonic schizophrenia (one of the types of schizophrenia), and organic disorder named organic catatonic disorder (4,15). The new diagnostic grouping for catatonia in the MBND chapter was implemented due to the significant association between catatonia and various mental disorders such as, bipolar and depressive disorders (20). As a result, catatonia is now an entirely new diagnostic group that is at the same hierarchical level as anxiety and fear-related disorders (1).

Bipolar Type II Disorder

Bipolar disorder (BD) is described as an episodic mood disorder classified by different manic, mixed, or hypomanic episodes. Such episodes tend to alternate with depressive episodes along the course of the disorder (2). The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) introduced two types of bipolar disorder. Type 1 BD is characterised by at least one manic episode. Type 2 BD is characterised by at least one hypomanic episode with at least one major depressive episode, and an absent history of manic episodes (4). Conversely, this threshold was listed as two or more episodes in ICD-10. The symptoms and diagnostic criteria of these episodes are summarised in figure 3. Notably, symptoms of mania and hypomania are similar. However, manic episodes are more severe and dysfunctional, resulting in more occupational, educational and social difficulties (21). Manic episodes may also include psychotic symptoms, such as grandiose delusional thinking.

Evidence suggesting the distinction between the two types of BD report differences in anti-depressant monotherapy (22), neurocognitive measures (22,23), genetic effects (22,24) and neuroimaging results (22,25,26). As a result, ICD-11 also subdivided BD into two types further enhancing clinical utility (27). However, whilst ICD-10 had already sub-divided BD and listed type II BD under 'Other bipolar affective disorders', ICD-11 harmonises with the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), and allows type II BD an equal status as type I BD (28).

Body Dysmorphic Disorder (BDD)

Individuals with BDD are constantly preoccupied with defects in their physical appearance that would otherwise be slightly noticeable or unnoticeable to others (11,29). The diagnostic criteria of BDD include the aforementioned preoccupation along with excessive self-consciousness about the perceived flaw (16). These are severe enough to cause significant distress or personal, social, and occupational impairment. Symptoms include excessive and repetitive actions to assess, hide and alter the perceived physical flaw (4). Presentation is relatively similar across age groups but its course and severity, are usually worse when the disorder's onset occurs in young people. Notably, such individuals are at increased

risks of suicide and other mental comorbidities (16).

BDD was initially known as "dysmorphophobia" in the third revised edition of the DSM (DSM-III-R) and then incorporated with hypochondriasis in ICD-10. In the latter, clinicians were instructed to diagnose it as delusional disorder if the patient experiences delusional thoughts. This implied that this disorder can fall under a different diagnosis in view of such delusional beliefs. As a result, ICD-11 included BDD as part of the obsessive-compulsive and related disorders (OCRD) chapter due to the similarities present (4). As shown in figure 4,

Table 2: Symptoms of increased, decreased and abnormal psychomotor activity diagnostic of Catatonia. Adapted from ICD-11, 2022.

Decreased		Increased		Abnormal	
Staring	Fixed gaze, decreased blinking, often with widely opened eyes	Extreme hyperactivity	Reason is absent	Rigidity	Increased muscle tone to produce resistance
Ambiten-dency	Appearance of a 'motorically stuck' movement of indecisiveness or hesitation	Extreme agitation	Reason is absent	Mannerisms	Odd, purposeful movements inappropriate to an individual's cultural context
Negativism	Behaviour opposing instructions leading to withdrawal from interaction with others	Combativeness	Striking out against others usually in an undirected manner with or without the potential for injury	Posturing	Spontaneous and active maintenance of a posture against gravity
Stupor	Markedly reduced mobility or immobility and minimally responsive to external stimuli	Impulsivity	Sudden engagement in inappropriate behaviour without an initial provocation	Stereotypy	Repetitive, non-goal-directed motor activity (finger-play)
Mutism	very little (unintelligible, hushed, whispered	Nonpurposeful movements	Reason is absent	Grimacing	Distorted or odd facial expressions that are often inappropriate or

	speech) or absent speech				irrelevant to the situation
/	/	/	/	Echophen-omena	Mimicking examiner's speech (echolalia) or movements (echopraxia)
/	/	/	/	Verbigeration	Continuous and directionless repetition of words, phrases or sentences
/	/	/	/	Waxy flexibility	Slight and even resistance to positioning by examiner
/	/	/	/	Catalepsy	Passive induction of a posture which remains held against gravity

Table 3: Symptoms making up manic, hypomanic and depressive episodes. Adapted from Pruthi *et al*., 2021.

Manic or Hypomanic Episode	***Three or more of these symptoms:***	Abnormally confident and restless Increased activity, energy and agitation Exaggerated euphoria Decreased need for sleep Unusual talkativeness Distractibility Poor decision making
Depressive Episode	***Five or more of the symptoms:***	Depressed mood Marked loss of interest Weight loss without a significant cause Weight gain Decreased appetite

		Insomnia or excessive sleep Restless or slower behaviour Fatigue Feelings of worthlessness or inappropriate guilt Unable to think or concentrate Suicidal ideation, planning or attempts

ICD-11 now emphasises the patient's level of insight as opposed to the presence or absence of delusional thoughts. This refers to how aware the patient is of his excessive behaviours or false perceptions (16).

Table 4: Insight specifiers in patients with Body Dysmorphic Disorder. Adapted from ICD-11 (2022) (16).

Insight Specifiers:	***With fair to good insight***	***With poor to absent insight***
Disorder-specific beliefs	Potentially not true	True
Alternative explanation to their feelings	Accepted	Rejected
Insight in relation to anxious levels	Markedly varies	Remains absent

Olfactory Reference Disorder

Olfaction is the sensation of smell secondary to odorous substances in the environment (30). Clinically, olfactory reference disorder is not uncommon and mostly occurs in the 20-year-old age group (1,16). Such patients are persistently preoccupied with a belief that one is emitting a perceived foul body odour or breath, that is either slightly noticeable or unnoticeable to others (1). This perception usually drives patients to repetitively check their odour emission, seek reassurance and avoid social events (29). Similarly, to patients with BDD, such patients suffer significant distress in all relationships they are a part of as they tend to fear rejection or humiliation from others noticing the odour (16). Olfactory reference disorder is also classified according to insight level (16). This disorder was introduced in ICD-11 as part of the OCRD chapter due to the presence of intrusive preoccupations followed by repetitive behaviours (4).

Hoarding Disorder

Individuals with hoarding disorder have a chronic and progressive obsession to accumulate possessions, secondary to excessive acquisition or difficulty discarding items, regardless of their actual value (31). As a result, homes become cluttered, compromising those who live there. For example, family members might be unable to locate personal items, move freely or exit their home and may become unable to use home appliances (16).

Such individuals are often under-diagnosed, thus, arguing its need for inclusion in ICD-11 from a public health perspective (32). Moreover, it can be a manifestation of other conditions namely, schizophrenia or dementia (4). Yet, studies report significant evidence of

hoarding disorder as a separate, unique condition. Therefore, this was included for the first time in the OCRD chapter of ICD-11 (28,33). Similarly, the severity of the disorder depends on the individual's insight to his/her symptoms. However, ICD-11 also highlights certain anxiety-provoking situations such as forcing the individual to discard their possessions (16).

Excoriation Disorder

This disorder has also been newly listed in the OCRD chapter as it is characterised by persistent recurrent skin picking behaviour, resulting in skin lesions. Commonly, the face, arms and hands are the primary picking sites but most individuals pick at multiple body sites. This tends to hinder individuals from their daily functioning and episodes can be brief or longer throughout the day. Excoriation disorder commonly co-occurs with depressive/anxiety disorders and obsessive-compulsive disorder (OCD) (16). A common comorbidity of excoriation disorder is trichotillomania; a disorder characterised by repetitive hair pulling (34).

Their inclusion in the OCRD chapter has been a point of debate as this repetitive behaviour is not following the presence of cognitive phenomena such as intrusive thoughts or persistent pre-occupations (4). Yet, their inclusion is based on the similar phenomenology, patterns of familial traits and reputed aetiology (28,35).

Complex Post-Traumatic Disorder (PTSD)

Complex PTSD occurs after an event or series of events that prove extremely threatening or terrifying to the individual (trauma). Commonly, the exposure is repetitive, and from which escape is difficult or impossible. Such examples include individuals who suffered torture, abuse or domestic violence. The diagnostic criteria for complex PTSD include all three criteria of typical PTSD, as depicted in figure 5, in addition to persistent problems in affect regulation, defeated self-beliefs, shame or guilt and difficulty to sustain relationships. Notably, the startle reaction mentioned in figure 5, may be reduced in complex PTSD, as opposed to PTSD where it tends to be enhanced (16).

Its inclusion in ICD-11 was based on evidence reporting poorer prognosis and benefits of different treatment modalities as compared to PTSD patients (36). In fact, ICD-11 construes PTSD as a fear-based disorder with symptoms of fear reaction, avoidance, and hypervigilance. Complex PTSD individuals additionally suffer from self-organisation disturbances (37). This group replaced the ICD-10 category of "enduring personality changes after catastrophic experience" (38). Despite significant empirical findings, DSM-V rejected the inclusion of complex PTSD as a separate disorder (28).

Prolonged Grief Disorder (PGD)

PGD is a state of abnormally persistent and disabling responses to loss of a loved one (38). During bereavement, such individuals face a persistent and pervasive reaction of grief because they long for the deceased's presence. This is in turn, accompanied with intense emotional pain. Symptoms include sadness, guilt, anger, denial, difficulty accepting the death, inability to experience a positive mood and emotional numbness (16).

Table 5: The symptom profile of PTSD. Adapted from ICD-11, 2022.

Re-experiencing the traumatic event in the current time		***Deliberate avoidance of trauma reminders***		***Persistent pre-occupation of a heightened current threat***	
Manifestations	Vivid intrusive memories/images	*Manifestations*	Active internal avoidance of thoughts/memories related to trauma	*Manifestations*	Hyper-vigilance, enhanced startles
	Flashbacks		External avoidance of people, conversations, acitivites or situations related to trauma		
	Repetitive dreams/nightmares	/	/	*Adaptations*	New self-defence behaviours
Feelings involved	Overwhelmed	/	/	/	/
	Re-experience the same intense emotions of the trauma	/	/	/	/

Usually, this experience of grief response lasts at least six months and results in social, occupational, and emotional impairment (4).

The inclusion of PGD in ICD-11 was based on evidence highlighting significant boundaries between normal and severe bereavement. The latter was found to benefit from professional psychiatric services to overcome the persistently severe mourning (28,39). Conversely, DSM-V included a bereavement disorder with symptoms for at least 12 months after the death known as "persistent complex bereavement disorder" (28).

Binge Eating Disorder (BED)

Individuals with BED tend to live with recurrent binge eating episodes that usually occur once or more weekly for several months. A binge eating episode is characterised by loss of eating control, thus, the individual eats excessively or differently

than usual. As a result, the individual feels unable to stop or limit eating. Individuals might also wake up late at night to eat large amounts of food. Episodes are usually accompanied by guilt, disgust and distress (16). Unlike bulimia nervosa, episodes are not regularly followed by weight-gain prevention behaviours like, self-induced vomiting or strenuous exercise. Often it is associated with weight gain but this is not considered an essential criterion since, typical weight individuals can struggle with BED aswell (4).

Instead of ICD-10 diagnoses of other unspecified or specified eating disorders, ICD-11 introduced BED based on a 20 year history of studies suggesting its validity and clinical utility (1,28,40). Similarly, changes paralleled to those of DSM-V which also included BED after having described it as a research category in the Appendix of DSM-IV (28). The parallel changes of both ICD-11 and DSM-V include features reflecting a general loss of control or changed eating behaviours, both of which resonate as clinical diagnostic criteria among clinicians (41). Notably, ICD-11 guidelines eliminated the essential diagnostic criteria of abnormally large amounts of food taken in during a binge eating episode (28). This was due to increasing evidence reporting that the subjective experience of a sense of loss of control, is more important than quantity of food eaten during episodes (42,43).

Avoidant/Restrictive Food Intake Disorder (ARFID)

Patients diagnosed with ARFID live with abnormal eating behaviours causing insufficient intake of food quantity or variety to meet their nutritional needs. This involves a very narrow repertoire of food items and picky eating. As a result, such individuals experience weight loss, failure to gain weight as expected during childhood or pregnancy and nutritional deficiencies. This may make them dependant on nutritional supplementation or tube feeding. Conversely to anorexia nervosa, concerns about body weight or shape are absent in individuals with ARFID (4).

Reed *et al.* (2019) report the inclusion of ARFID in ICD-11 was an expansion of the ICD-10 category "feeding disorder of infancy and childhood" and additionally refers to adulthood (4,40). Given the limited research on its aetiology, prognosis and treatment, its inclusion in ICD-11 should provide guidance to clinical practice and novel research (28).

Body Integrity Dysphoria (BID)

This rare disorder is characterised by a persistent desire to have a specific physical disability such as, paraplegia or blindness. This usually presents in childhood or early adolescence and manifests in different ways, such as, fantasies of having the disability, engaging in "pretending" behaviour or spending time searching for methods to achieve the desired disability. As a result, such individuals are less productive and less participative in social situations. Unfortunately, a minority of individuals with BID pursue the desired disability via surgery or self-harm that demands surgery (4,16).

Notably, ICD-11 values BID and bodily distress disorder equally in the hierarchy but are categorised separately under "Disorders of bodily distress or bodily experience" (16). The distinction was important since bodily distress disorder is characterised by persistent bodily symptoms perceived as distressing thus, the individual directs excessive attention towards the symptoms. As a result, patients

may repeatedly present acutely to clinicians and investigations do not alleviate the patient's excessive pre-occupation (44).

Gaming Disorder

Gaming disorder is characterised by a pattern of persistent or recurrent gaming behaviour. Its manifestations are depicted in figure 6 and causes significant distress and functional impairment in daily life. The gaming behaviour can be continuous or episodic and recurrent for at least one year. ICD-11 sub-divides gaming disorder in predominantly online and predominantly offline types. Evidently, online forms are reported more problematic (45). Such individuals often experience urges to engage in gaming behaviour during other activities, and upon gaming cessation individuals tend to experience dysphoria, and exhibit aggression (4,16).

Figure 6: Manifestations and examples of each manifestation of gaming disorder. Adapted from Reed *et al*.,2019, and ICD-11, 2022.

Manifestation	Example
Impaired control over gaming behaviour	Unable to limit time spent gaming
Increasing priority to gaming over other life interests and daily activities	Rather be gaming than socialising
Continuation or escalation of gaming behaviour despite negative consequences	Repeatedly fired from work secondary to absences due to gaming

Given that recent technological advances have attracted more individuals to computer sets, excessive gaming behaviour is becoming increasingly common. As a result, ICD-11 added gaming disorder with "disorders due to addictive behaviours" along with gambling disorders (4,45). Yet, its inclusion initiated numerous debates among clinicians arguing scientific basis of gaming disorder is too weak or non-problematic gamers can now be stigmatised by its inclusion (28,46,47). Additionally, utilising the internet as a diagnostic specifier was a new approach in both gaming and gambling disorders. Moreover, "hazardous gaming" and "hazardous gambling or betting" were defined to create alternatives for individuals who do not meet the diagnostic criteria for the respective disorders. Its inclusion paves the way for potential interventions (28).

Compulsive Sexual Behaviour Disorder (CSBD)

Individuals with CSBD persistently fail to control intense repetitive sexual urges resulting in repetitive sexual behaviour for at least six months. As a result, such individuals are socially, personally, and occupationally impaired. This usually manifests as neglect of other interests and personal health to prioritise one's sexual urges. Numerous cessation attempts are unsuccessful, and this behaviour persists or escalates despite its limitations. CSBD individuals tend to continue such behaviour even when they no longer derive pleasure from it (4,16). Stein *et al.* (2020) reports CSBD is likely to have various aetiologies thus, ICD-11 did not focus on these (28). Notably, the sexual behaviour in CSBD is not considered a true compulsion secondary to intrusive preoccupations, but secondary to an initially rewarding behaviour that gives the patient pleasure. This turns into uncontrollable behaviour of both compulsive and impulsive nature (28,48).

CSBD was introduced in ICD-10 "excessive sexual drive" category. This never clearly described CSBD but highlighted nymphomania and satyriasis. Despite its phenomenology resembling behavioural addictions, CSBD is included in ICD-11 with impulse control disorders. This is because evidence confirming the disorder's development and maintenance being similar to those in substance use disorders, is absent. Yet, its inclusion in ICD-11 paves the way for newer treatment and potentially reduce the stigma and distress for patients (4,49).

Intermittent Explosive Disorder (IED)

Individuals with IED live with repeated brief episodes of aggression or destruction of property. Aggression can be verbal or physical and represents lack of aggressive impulse control. These manifest as intense outbursts which are not congruent to the initiating provocation. This only meets diagnostic criteria if episodes are not better explained by another psychiatric condition since, aggressive episodes can also be due to oppositional defiant disorder, conduct disorder, and BD, among others (4,16).

IED was introduced in DSM-III-R then, in ICD-10 as an inclusion term in "other habit and impulse disorders" (15). However, ICD-11 included this under impulse control disorders based on evidence supporting its validity and clinical utility (50). Other impulse control disorders include pathological gambling, kleptomania, pyromania and CSBD (51).

Premenstrual Dysphoric Disorder (PMDD)

PMDD is characterised by various severe mood, cognitive and somatic symptoms. These symptoms usually begin several days before onset of menses until they improve during menstruation and stop within one week after the onset of menses. Diagnostic criteria include a pattern of mood-related symptoms namely, depressed mood or irritability, somatic symptoms such as lethargy and joint pain or cognitive symptoms, like forgetfulness or concentration impairment. Individuals with PMDD experience such symptoms during most menstrual cycles within the past year and they are severe enough to impair their functioning socially, educationally and personally (1,4).

Introduction of PMDD in the research appendices of DSM-III-R and DSM-IV stimulated various research to establish validity of PMDD (52,53). Hence, both ICD-11 and DSM-V included PMDD as a separate condition. Despite ICD-11 listing it as part of genitourinary diseases, it was cross-listed in the category of depressive disorders due to the prominence of mood symptomatology (4,16). Notably, PMDD was distinguished from premenstrual tension syndrome. The latter being less severe with less impact on daily functioning (52).

Conclusion

Evidently, the new additions to the MBND chapter of ICD-11 have proved to be the first major revision of the world's foremost mental disorder classification in nearly 30 years. The new disorders along with other significant changes in the pre-existing mental disorders,

have involved unprecedented efforts from global representatives and clinicians. As a result, participation was multi-disciplinary and multi-lingual. Notably, ICD-11 and its approach emphasise that the science behind disease classification (nosology) is not the solution to all debates in psychiatry and that nosology itself is not necessarily flawed. The ICD-11 and its focus on clinical utility and global applicability increases the likelihood of implementation by mental health clinicians and non-specialist settings alike.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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Anatomical and Pathophysiological changes of the musculoskeletal system due to Obesity

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Abstract

Obesity is a growing global epidemic affecting all ages. Obesity has wide ranging negative effects all over the body. This literature review aims to show the effects of obesity on the musculoskeletal system in children and adults. A literature review was carried out by finding studies on the database PubMed to base the review upon. Some inclusion and exclusion criteria were set, based on which the articles were chosen. The types of studies that were included were cross-sectional studies, literature reviews, case control studies and random control study. There are both anatomical and pathophysiological changes in obesity in all the organs and organ systems reviewed. It was concluded that obesity has local changes such as bone deformity and systemic changes since the musculoskeletal system has an endocrine function and obesity interferes with the secretion of the biomolecules from the organs. It was noted that obesity has long lasting negative effects on the musculoskeletal system, so it would be better to focus more on the prevention of obesity with regular exercise and a healthy diet.

Keywords: Obesity, Musculoskeletal system

Introduction

Obesity is defined as an excessive accumulation and storage of fat in the body. Obesity can be determined with the help of the Body Mass Index (BMI). When BMI is greater than 25, one is considered overweight and when one has a BMI greater than 30, they are considered obese. Body Fat Percentage (BFP) and Waist to Height Ratio (WHtR) can also be used to measure obesity (1).

According to a study by Cuschieri et al., the Maltese population has an obesity problem, with 34.1% of adults being obese. In adult males, 36.89% are obese, while 31.25% of the adult females of the population are obese. In addition, a staggering 69.75% suffer from an

abnormally high body weight. It was also reported that there is a variation in the rates of obesity amongst people of different ages. Specifically, males between 34-44 years had the highest percentage of obese individuals amongst themselves and females between 55-64 had the highest percentage of obese individuals amongst themselves. (2).

The anatomical changes occurring as a result of obesity will be explored by considering the changes in the bone development of children with obesity. The changes in fracture healing and bone healing due to obesity will be reviewed and the shifts in the positioning of bones and their rotation in their anatomical position due to obesity will be discussed. The pathophysiological changes occurring as a result of obesity will be noted by loss of bone mineral, and a change in the composition of the bone marrow. Additionally, the levels of bone derived factors and the changes in the levels of inflammatory cytokines in fractures due to obesity will also be reviewed.

This literature review aims to bring awareness on the impact of obesity on the musculoskeletal system, with the hope to encourage individuals to deal with an obese status by having an understanding of some of the underlying pathophysiology complications.

Methodology

Table 1 shows the steps that were taken before the start of the writeup to find the necessary articles on which to base this literature review on.

Results and Discussion

Loss of Bone Mineral Density in obesity

Depending on how obesity is defined, whether it is according to a change in BMI or PBF, a change in BMD was noted. With regards to BMI, the BMD increased but with regards to PBF, BMD decreased (3,4).

In obese females, defined by BMI, reduced loss of BMD at the lumbar spine was noted (5) it can be stated that obesity defined by BMI, reduces chances of vertebral fractures (6). However, it was also noted that obesity defined by PBF increased the chances of fracture(6). With regards to BMD in the total hip and femoral neck it was noted that there was no significant effect (5). Additionally, no change has been noted in whole body and total hip BMD, based on differences in BMI. However, in older obese people of both black and white ethnicities, a greater BMD loss from the femur was noted when compared to normal individuals (5). Increase of visceral adipose tissue, thus increase in PBF, lowered BMD and had negative effects on bone health . Even though a lot of studies showed an increase in BMI, increase BMD, as stated previously, some studies did not support this and another study found that this was not the case for African Americans women (3,8). The above stated relationship between obesity and BMD is applicable for males but in females it did not apply due to the presence of high amounts of oestrogen and a significant drop in it after menopause. Hence why the relationship between obesity and BMD in females changed with age and at the start of menopause (3). There was a difference in results in BMD loss with obesity, some claimed higher BMI decreased loss of BMD and others found it increased the loss of BMD.

Changes in bone derived factors due to obesity

Visceral obesity raised three bone derived factors in body namely, Serum Osteocalcin (OCN), Fibroblast Growth Factor (FGF23) and Neutrophil Gelatinase Associated Lipocalin (NGAL). These secreted factors were independent, and did not affect each other. The above mentioned factors regulate different pathways with regards to regulation of energy metabolism in the body (9). Function of OCN and FGF23 included regulating energy metabolism (10). Circulating OCN and monocytes was elevated in vascular disease so could be used as a marker in diabetic patients (11). When Visceral Fat Area (VFA) increased, serum OCN decreased, as they are inversely proportional (12). Those with obesity, especially visceral obesity, were noted to have a higher level of FGF23 and NGAL. So, a higher level of these two and a lower level of OCN in the serum could be used as a marker to detect obesity (9). OCN also has a protective function against obesity so a lower level of it could lead to an increase in adiposity and obesity while when it rises, it would lower the PBF (13). The PBF is reduced since OCN promotes division of adipocytes and increases production and activity of adiponectin (14). Adipokines were

Table 1: Analysis of the methodology used to find the relevant articles used for this literature review. It includes the number of articles found under each subsection and then an explanation of how the desired articles were chosen.

Library sources used to perform the literature research	PubMed Database				
Time frame of publication of included studies	01/01/2018 to 31/12/2019				
Types of studies considered	International studies, cross- sectional studies, random control trials, registry based studies, literature reviews, longitudinal studies, case control studies, cohort studies and biopsy based studies				
Keywords used	**Obesity [Title]**	**(bones) AND (obesity)**	**(obesity) AND (fracture)**	**(BMD) AND (obesity)**	**(Malta) AND (obesity)**
Number of matched articles	11,392	498	395	175	153 (no restriction for time frame in this one)
Process of selection of articles that would be used in the review	Selection of papers done by first reading the abstract. If a research topic is not relevant, then the paper is eliminated. After this the discussion and results sections of the remaining papers are read. This becomes the final criteria of whether the paper will be used in the review or not.				

noted to trigger a feedback loop which stimulated expression of FGF23 in the bones, thus maintaining a suitable adipose tissue level (15).

Long term effects of obesity on the musculoskeletal system at different ages

Obesity affects people of all ages, even children. Children suffering from obesity are more likely to have fractures, muscle pain, lower limb extremities misalignment, change in hip range motion and joint pain (16). The longer the children spend in an obese state, the more changes they would have in their range of hip motions. Slipped Capital Femoral Epiphysis (SCFE) is a pathology related to obesity in teenagers, where their femoral head slips off the neck of the femur to be displaced posteriorly (17) . Approximately 81% of the SCFE patients were obese in a study by Manoff and Nasreddine et al. IIf the obesity was maintained in the child and the weight was not returned to a normal BMI, there was a 3.5 times higher chance that the other limb, the normal one, would also have SCFE eventually (18). When obesity is combined with mild lower extremities malalignment, there was a greater chance of suffering from a deformity on the upper part of the femur as well. When considering hip motions, obese children experienced a greater change in all the different types of motion, greater external rotation and decreased internal rotation (17) .

Obese individuals were more likely to have chronic pain in the back, legs and feet. In a survey conducted in China, obese elderly individuals reported 71.7% prevalence of chronic pain when compared to normal weighing elderly people. Popular to contrary belief, age was not a factor in this case. The most common location for chronic pain in elderly was reported to be within the legs or feet. Some reports hypothesise the pro-inflammatory activity of the adipose tissue might be the cause behind the chronic pain (19). For example, the presence of higher levels of C-Reactive protein (CRP), which is a pro-inflammatory marker, increased the chances of chronic lower back pain in obese patients (20). Besides the inflammatory reaction of the adipocytes, there were also structural changes in an obese individual, such as change in lumbar disk height in L1-L4 vertebrae, which can lead to back pain. However, it is important to note that there was no change in the lumbosacral joint (21). However in sarcopenic obesity, which is low muscle mass in combination with increased adiposity in the elderly, there was an increase in risk of osteoporosissince the muscle mass decreases and PBF increases (22). Sarcopenic obesity also increases fracture risk when compared to obesity alone due to poor quality muscle mass which reduces bone strength and balance (23). The risk of fracture in obese people is noted to be a controversial topic as different studies show different results. Some claim risk of fracture increases with obesity, others claim it decreases and the results vary even on where the risk of fracture is being assessed as seen in Table 2.

Effects of obesity in fracture repair

For a fracture to heal, a callus needs to form at the site of the fracture. This callus is filled with cartilage. The cartilage is then absorbed and replaced with new bone (30). Fracture repair in obese mice was noted to take longer, since there was lesser volume of callus formed and there were no continuous cortical bones at the fracture site. This could also be observed in the histological slides of the bones. However, in a fracture in normal mice, there was trabecular bone at the fracture site which was absent in obese mice. In obese individuals it was noted that there was more bone callus than cartilage callus at fracture site. Obese people also had slower cartilage formation at the fracture site than normal individuals (31).

Mesenchymal cells and chondro-osteoprogenitor cells need to be recruited to the fracture site for quick healing but the rate at which they are recruited in obese individuals is slower than normal weighing people (5,32).

Table 2: Different studies showing different results in risk of fracture in obese individuals compared to normal weighing individuals.

Upper Limb	Lower Limb	Reference
↓	↓	Ip et al., 2009
↓	↑	Compston et al., 2011
↑	↔	Ma et al., 2011
	↑	Shah et al., 2011
	↑	Pace et al., 2001

The role of the mesenchymal cells is to differentiate into osteoblast. This differentiation requires growth factors such as Calcitonin Gene Related Peptide (CGRP), FGF and Transforming Growth Factor (TGF-β1) (21). CGRP is a protein that is released from brain tissue to heal fractures and this can be proven because this protein was found in higher concentrations in individuals with traumatic brain injury (33). Presence of FGF-2, released from tissues, increased bone formation in mice, so a high amount of it speeds up the process of callus remodelling, cartilage, and bone formation but a low amount of it, which was seen in obese people, slows fracture healing (31,33). In fracture healing in mice, levels of TGF-β1 first increased and then decreased in the plasma. However, in obese mice, first the levels of TGF-β1 decreased and then increased slightly and then started to decrease again. So their levels remained lower than those of normal individuals. Lower levels of FGF and TGF-β1 as seen in obese people, have slower blood vessel formation at site of fracture which means less chondrocytes and mesenchymal cells reach the area. Their low levels also mean that the rate of differentiation from mesenchymal cells to osteoblasts is slower which means that less osteoblasts were present at the site of fracture. Fracture of the bone triggers an inflammatory response which means that levels of Tumour Necrosis Factor (TNF-α) increased in the blood plasma and remained relatively high, especially in obese individuals for whom it takes longer for fracture healing (31).

Bone health in obese individuals

Better bone health was present in individuals with a higher Appendicular Lean Mass (ALM) and higher proximal tibial volumetric BMD (vBMD) has been associated with lower leg muscle density. Proximal tibial cortical vBMD and area had a positive correlation with lower leg muscle density and cortical thickness in obese females. Another correlation of lower leg muscle density was that the higher the density, the poorer the balance in sarcopenic obese individuals. In obese women, this was negatively correlated with postural sway in sarcopenic obesity (23). It was noted that there was a decrease in areal BMD in sarcopenic obesity in old men compared to those with normal obesity (35). Studies have shown that higher muscle mass improved bone health in old adults (23). Fat infiltrating the muscles had a negative effect on bone health and if this was reduced in older women, their bone strength has been noted to have increased. The negative effect was due to lipotoxicity and local inflammation (36). People with high BMI have been noted to have higher proximal tibial Strength-Strain Index (SSI) and whole-body areal BMD (aBMD). Women specifically with high BMI have been observed to have greater cortical

area and thickness. However, specifically in males, they had a lower cortical vBMD when obese. Bone strength and size were improved by higher lean mass not fat mass (23) and in obese individuals, there was a difference in bone metabolism due to the over production of leptin (37).

Study Limitations

Some of these changes are observed in experiments conducted in mice which might not necessarily correlate with humans. Additionally, there are a lot more effects that obesity has on the human body that were not discussed in this review. This review also did not focus on the genetic susceptibility of a person to obesity, thus ignoring the fact that all the effects of obesity vary from person to person depending on their genes. This review also does not provide solutions to any of the problems mentioned as it is meant to raise awareness about the effects of obesity on the musculoskeletal system. Additionally, research covered only a limited age band and excluded different ethnicities, such as Asians, Arabs and Hispanics and age groups, such as middle aged adults. There were also discrepancies on how different researchers categorised obesity which could have affected the study's interpretation.

Conclusion

The prevalence of obesity has been growing worldwide over the last decade and is now an epidemic. This increase has been shown in all age groups and in a wide variety of communities, both within the low income and high income communities (38).

As discussed throughout the review, obesity causes a lot of anatomical and physiological changes in the musculoskeletal system. Some of these changes have been shown to be reversible but a vast number of them are not, especially when the changes take place in children. Additionally, when obesity attacks an organ system, it does not only alter the anatomy but it also alters the pathophysiology. This change, as discussed, affects the musculoskeletal system, which has far outreaching effects to other parts of the body, including the endocrine system. Therefore, due to such drastic changes in both the anatomy and the pathophysiology as a result of obesity, it is important to keep in mind the ccccimportance of healthy eating and controlling weight gain, especially if the weight gain starts early on in life (1).

Declarations

Conflict of interest: N.A.

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Post hair removal folliculitis; a clinicopathological evaluation

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Abstract

Folliculitis refers to the process by which a hair follicle becomes inflamed. This has a number of aetiologies, complications and clinical implications. Hair removal is a common cause of folliculitis. People who make use of depilation techniques are at an increased risk of developing folliculitis. Should they have other factors that further depress their immunity, complications are more prevalent. These include spread of infection, the formation of furuncles, permanent scarring, hyperpigmentation and the destruction of hair follicles. This takes considerable psychological toll on the involved individuals. In order to prevent these complications from arising, as well as ensuring full recovery, it is imperative that treatment given is taken for the prescribed duration and in the correct form. Antibiotics are the mainstay of treatment when folliculitis is due to *Staphylococcus aureus* infection. In severe, recalcitrant cases especially, a dermatologist should be consulted to ensure optimal care. The safest way to prevent this condition from developing is making sure cleanliness is a priority, both regarding any tools used as well as the condition of the skin prior to subjecting hair follicles to any particular treatment.

Keywords: Folliculitis, Hair Removal

Introduction-What constitutes a hair follicle?

Hair exerts several functions in mammals, of whom it is a primary characteristic. Among these, there are thermoregulation, proprioception, physical protection, as well as social interaction. It plays a major aesthetic role in humans as opposed to the intrinsically necessary role it plays in cats, for instance, when it comes to scaring off predators (1).

The hair follicle, a skin appendage located deep in the dermis is in fact one of the most delicate complex organs in the body. The pilosebaceous unit of a hair follicle is composed of the follicle itself along with an adjacent sebaceous gland and erector pili muscle. Sebaceous glands are holocrine

glands, especially numerous in certain areas of the skin such as the face, scalp, chest and back. These glands open directly onto the hair follicles in most instances, excluding areas like the lips, where they instead empty directly onto the mucosal surface because the lips do not harbour hair follicles (2). There are three different phases of the hair growth cycle, these being the anagen (growth) phase, the catagen (transitional) phase and the telogen (resting) phase (3).

What is folliculitis?

Folliculitis refers to the process by which hair follicles become inflamed. While this condition is oftentimes self-limiting, prompt recognition and treatment are necessary in order to improve the quality of life of affected patients (4). Usually, folliculitis is caused by a superficial or deep bacterial infection of the hair follicle. However, this does not exclude the possibility of infection by fungal species like *Malassezia* yeasts or even viruses, such as Herpes Simplex virus (5).

Folliculitis affects the skin around the respective hair follicles, usually leading to a tender inflamed pustule (5). There are two types of folliculitis; superficial- affecting only part of the follicle, as well as deep which occurs when the whole hair follicle is damaged. Symptoms vary depending on which type of folliculitis and its severity (6). Bacterial folliculitis is the most common superficial form, mostly arising due to Staphylococcal infections, leaving the skin itchy, with small pustules (7).

Folliculitis decalvans (FD) is a rare condition leading to chronic inflammation of hair follicles. This classically presents as peripheral pustules surrounding an expanding patch of alopecia. Pruritus and pain may also occur. FD results in follicular destruction and permanent hair loss. The mainstay of treatment is antibiotic therapy, as the chief causative agent is most commonly *Staphylococcus aureus* (7).

Who can get folliculitis?

Bacterial folliculitis can affect children and adults alike. Most forms do not have a predilection for race, age and sex however some do. Factors which predispose to folliculitis include frequent hair removal practices, be it shaving, waxing or other forms of epilation. Tight clothing might also cause an abrasive environment, leading to friction which irritates the hair follicle (8). The use of topical corticosteroids can also predispose to bacterial folliculitis due to systemic immunosuppression (9).

Hot tubs or pools that are inadequately cleaned should be avoided as they increase the risks of developing folliculitis (9). This may predispose to what is known as a "hot tub rash". In this case the culprit organism is *Pseudomonas aeruginosa* causing a Pseudomonas folliculitis. The latter is characterized by follicular papules, vesicles and pustules which may be crusted. These sorts of lesions preferentially affect areas covered by swimwear, such as the buttocks and usually spare the face, neck, soles and palms (10).

Why hair removal?

Hair removal practices, being so customary, especially in certain areas of the world, are

one of the leading causes of folliculitis. In today's world, the list is quite extensive, ranging from shaving, plucking, depilatory creams, hot waxing, threading, electrolysis as well as laser hair removal (11). Although some methods might be preferred over others due to the pain tolerance level of the individual as well as the cost, easily accessible means of hair removal like waxing are used much more commonly, when compared to electrolysis. The risk of folliculitis with waxing is much higher, however, than with other forms of hair removal (12).

Among the issues that can arise due to hair removal there is skin inflammation, minor burns, scarring as well as hair follicle infections. If folliculitis isn't treated adequately, it can also result in permanent hair loss and scarring (12). If one is prone to folliculitis, laser hair removal can help prevent future outbreaks. On the other hand, practices like shaving with a blade, as well as waxing predispose to folliculitis much more. Waxing is in fact discouraged in patients at high risk or suffering from recurrent bouts of folliculitis (13). Given that different hair removal practices have different risks for folliculitis, one should weigh the pros and cons of every method before complying to any one in particular (14).

A rather specific process by which electric current is used to remove hair roots or small blemishes on the skin is called electrolysis (14). The latter is a hair removal treatment carried out by a trained electrologist which destroys the hair follicle permanently, thanks to a thin wire passed into the hair follicle under the skin surface. When performed safely by a trained electrologist, this process confers more permanent results than laser or waxing treatments. Folliculitis can also result as a complication of this painful and time-consuming procedure. This non-infectious type of folliculitis arises due to the fact that hairs start to regrow after being removed, resulting in an irritated hair follicle and surrounding inflammation (15).

Management of post hair removal folliculitis

Firstly, preventative measures should be taken in order to minimise the risks of developing folliculitis after hair removal, regardless what the method opted for is. These measures include regular exfoliation (16).

In physical exfoliation, a coarse brush or washcloth, often together with a scrub is used to slough off dead skin cells as well as other debris (16). Chemical exfoliation uses a gentle chemical exfoliant like salicylic acid that can do the same thing without abrading the skin so much. This might reduce the risks of getting folliculitis in the long run, as through exfoliation the hair follicle is getting rid of the excess sebum and dirt, both of which are wonderful substrates for bacteria and other micro-organisms. Cleansing is also important, both before and after epilation (17). If one carries out rigorous physical activity after hair removal, it is imperative to cleanse again within an hour of this (18).

Apart from the state of the skin follicles before the procedure, it is also worthwhile focusing on the method and tools used to shave, as these too can play a major role in avoiding folliculitis. Having clean tools, shaving with a sharp, clean blade and never sharing razors with anyone else is crucial to avoid unwanted consequences. It is also useful to shave in the direction of hair growth,

after applying shaving cream or gel. A razor should be stored in a dry area, and not in the shower, where it can accumulate bacteria and even get rusty, which further increases the potential for infection (18).

The treatment of post hair removal folliculitis depends on the extent and spread of the inflamed hair follicles, as well as the severity. Certain types of folliculitis may need more aggressive treatment regimens while others might resolve spontaneously (19). If folliculitis is mild, antiseptic cleansers can be used to clean the skin and warm towels can be applied to irritated skin to soothe the discomfort. In more severe forms of folliculitis, oral antibiotics may be needed to treat the condition. Topical antibiotics may also be used (20).

Superficial folliculitis normally improves within 7 to 10 days. If no improvement is seen within this time period, one should reach out to a doctor. In the case of deep folliculitis, medical intervention is often required, and these patients should be referred to a dermatologist. Most cases may be treated with an oral anti-staphylococcal antibiotic such as flucloxacillin, or if penicillin-allergic, a tetracycline. In the case of severe pain or itching, NSAIDs or antihistamines can be prescribed. Antibiotics and other medications may not clear up chronic cases of folliculitis. If the latter is ongoing and especially more so in an acute flare up, hair removal should be avoided. Although laser hair removal may prevent folliculitis from developing, it cannot be used to treat it (21).

Apart from treating the infection while it is rampant, one should also make sure to minimise exposure to other harmful practices that might hinder skin healing. It is imperative to not touch or pick at pustules, use fresh towels every time the face is washed and not share towels with anyone else. Perfumed products should also be avoided on the affected areas. Excessively hot water should also be avoided as this can irritate the already inflamed skin. Until the infection clears up, one should also abstain from using swimming pools, saunas and hot tubs as well as carrying out any forms of activity that makes sweating excessive (22).

Complications of folliculitis

Without adequate treatment and in an underlying immunosuppressive state, bacterial folliculitis may progress to pus-filled painful lumps known as furuncles. A cluster of these furuncles is known as a carbuncle. These form a connected area of infection underneath the skin (23).

Generally, the treatment of these conditions involves the application of warm compresses to relieve pain and promote natural drainage, however incision and drainage might also be indicated. In such cases, the infection would have spread deeper around the hair follicles. Antiseptic cleansers and oral antibiotics are definitely useful in such situations (24).

Other complications include scarring, dark patches, permanent hair loss due to the damaged follicles, the recurrence of folliculitis as well as cellulitis (25).

Conclusion

In conclusion, folliculitis, a common skin condition in which the hair follicles become

inflamed, is one of the consequences of hair removal; some methods having a larger predisposition than others. Although there are ways to minimise the extent of this inflammation, when it happens treatment should be started as soon as possible to prevent the development of complications that can follow suit (26).

Declarations

Conflict of interest: N.A.

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Dilated Cardiomyopathy

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Abstract

Dilated cardiomyopathy (DCM) is a heart disease that enlarges the heart muscle and reduces its function, leading to heart failure, arrhythmia, and sudden cardiac death. The causes of DCM are not always clear, but may include genetic mutations, viral infections, alcohol abuse, and certain medications. Common symptoms include shortness of breath, fatigue, swelling, and irregular heartbeats. Diagnosis involves physical exams, ECGs, echocardiograms, and additional tests to identify underlying causes. Treatment includes medication, such as ACE inhibitors and beta blockers, to improve heart function, diuretics to reduce fluid buildup, and anticoagulants to prevent blood clots. Surgery or implantable devices, like pacemakers or defibrillators, may be necessary. Early diagnosis and treatment are crucial for managing DCM, and many individuals with DCM can lead full and active lives with proper treatment and lifestyle changes.

Introduction

Dilated cardiomyopathy is a myocardial disorder characterized by progressive ventricular dilatation and myocardial stretching, impairing systolic function. This, along with valvular regurgitation, gives rise to congestive heart failure (1).

DCM constitutes 90% of all cardiomyopathies. 25-30% of DCM can be identified as familial (2), however the recent increase in incidence rates in the younger population is suggestive of non-genetic factors contributing to the development of the disease in predisposed individuals (3). Apart from genetic predisposition, the etiology also includes malnutrition, cytotoxic agents such as anthracycline derivatives, viral myocarditis and autoimmune disease (4).

The diagnosis of idiopathic DCM is based on:

1) a left ventricular ejection fraction of less than 45% and/or fractional shortening less than 25% and
2) end-diastolic diameter greater than 117% of the predicted value based on age and body surface area.

Familial DCM is diagnosed if there are two or more affected relatives or by the sudden death of a first-degree relative before reaching the age of 35 (5).

Mild cases of DCM can be totally asymptomatic but undiagnosed, it may become symptomatic, or even fatal (6). Symptoms of DCM are due to cardiac inadequacy – angina, lethargy, dyspnoea and oedema as a result of congestive heart failure (7). The prognosis of DCM is dependent on a multitude of factors, namely the cause, degree of disease and functional impairment and comorbidities. In the United States, it accounts for approximately 46,000 hospitalisations and 10/000 deaths annually (8). Moreover, a recent study showed that 8% of Sudden Cardiac Deaths are caused by DCM (9).

The aim of this review is to give a brief overview of the pathophysiology, detection and management this devastating disorder, which can be screened for and essentially prophylactically managed, minimising its adverse outcomes.

Causes

Genetic

Genetic mutations in genes encoding for sarcomere, cytoskeletal and envelope proteins account for 20-48% of cases (10).

Z-discs are the basic contractile unit of the cardiomyocyte, approximating when there is cardiac muscle contraction. Along with the cytoskeleton, they are vital for the structural integrity, contraction and mechano-sensing transductions of the cardiomyocyte. Titin is an elastic protein anchored in the Z-disc. It is passively stretched in diastole, returning to its original size once contraction has occurred. It is coded for by the gene *TTN*, present on chromosome 2. Non-sense or frameshift mutations in the *TTN* gene, cause truncation of the protein, interfering with its elasticity. Such mutations are responsible for up to 25% of DCM cases, with an even higher prevalence in patients over 40 (11). Formatting...

Mutations in the *DES* gene, which encodes for desmin, a cytoskeletal protein forming muscle-specific intermediate filaments account for 1-2% of dilated cardiomyopathies (12).Formatting...

Dystrophin, encoded for by the *DMD* gene on the X chromosome, is important in the transmission of force of contraction, being the mechanical link between the intracellular cytoskeleton of all muscles and the extracellular matrix. DCM arising from *DMD* mutations were linked to 90% of Duchenne's muscle dystrophy cases and 70% of Becker's muscular dystrophy cases. However, isolated DCM can exist without signs of muscular dystrophy (13).

Desmosomes hold myocardial cells together, ensuring the mechanical and electrical integrity of the heart. An autosomal dominant pattern of inheritance of desmosomal genes has been noted in patients suffering from DCM. The *DSP* gene encodes for the desmoplankin protein, an intracellular component of desmosomes. *DSP* gene mutations result in DCM associated with left ventricular fibrosis and ventricular arrythmias (14).

The sarcomere is the functional unit of the cardiac muscle consisting of thin actin and thick myosin filaments. Mutations in the genes encoding for these proteins give rise to 10% of cases of familial DCM while

mutations in *MYH7* gene, encoding for the myosin heavy chain beta subunit are associated with 4-6% of familial DCM cases.

Acquired

Acquired forms of DCM may result from; myocarditis, sarcoidosis, toxins like alcohol and drugs, endocrine and metabolic disturbances and pregnancy.

Myocarditis damages the heart muscle by scarring, limiting its ability to contract. It is brought about by acute phase viral inflammation where exposure of intracellular antigens leads to a T-lymphocyte-mediated inflammatory response which may persistent in some patients with with poor immune response. Around 20% of myocarditis patients develop chronic DCM (6).

In sarcoidosis, granulomas form in heart muscle tissue as a response to inflammation, possibly causing fibrosis, restricting the elasticity of the heart muscle, leading to left ventricular dilatation and loss of contractile power. The papillary muscle and mitral valve function may also be affected. Heart blocks and tachyarrhythmias commonly arise from the disease due to interference with the conduction pathway of the heart (15).

36% of DCM cases in the West are associated with alcohol abuse. It causes tachycardia and vasodilation and cardiac muscle hypoperfusion, weakening its contractility. Alcohol is pro-arrhythmic. By causing electrolyte abnormalities it affects the heart rate variability and the QT interval (16). Cocaine stimulates the sympathetic system, increasing myocardial oxygen demand and causing coronary vasospasm, leading to coronary micro-ischaemia and scarring. Therapeutic drugs such as doxorubicin, used in chemotherapy are possible culprits (17).

In hemochromatosis, iron is deposited in organs, causing damage and dysfunction. In the heart, it deposits in the ventricle initially, causing DCM, reducing the ejection fraction, and giving rise to arrythmias. Hemochromatosis is not entirely acquired since it can be caused by a mutation in the HFE gene which controls absorption of iron in the small intestine (18).

Peripartum cardiomyopathy is DCM in pregnancy. Although the underlying causes are still unclear, it is thought that it may be a result of a previous abnormal immune response or viral illness. It can be difficult to detect as its symptoms may be attributed to usual third trimester physiology such as lower limb oedema and slight dyspnoea. It is diagnosed when heart failure develops in the last month of pregnancy or within 5 months of delivery with no other possible cause and when the ejection fraction is decreased to less than 45% (6).

Pathophysiology

Ventricular remodeling is brought about by cardiomyocyte hypertrophy and apoptosis, proliferation of myofibroblasts and interstitial fibrosis. In DCM, this occurs because of myocardial injury, increased left ventricular stress and haemodynamic disruption, bringing about an increase in oxidative stress, endothelin and pro-inflammatory cytokines, upregulation of the renin-angiotensin-aldosterone system (RAAS) and the adrenergic nervous system. This results in pathological left ventricular remodeling, giving the ventricle a spherical shape (19).

Left ventricular function changes in conjunction with the alteration in shape and size. Though there is an increase in end-diastolic volume and preload, the stroke volume and cardiac output are reduced because the alteration of the extracellular matrix of the ventricles disturbs the excitation-contraction coupling, making it stiff. This ventricular stiffness also prevents complete relaxation of the ventricles during diastole, impeding ventricular filling (7).

$$\text{tension} = \text{pressure x } \frac{\text{radius}}{\text{wall thickness}}$$

In, DCM, according to the Law of LaPlace, the pressure in the left ventricle increases, increasing the wall tension. A larger force is needed to push the blood out of the heart. Repressed contractility reduces the force produced, resulting in a decrease in stroke volume, leading to organ hypoperfusion, including the heart itself. Insufficient energy supply causes deterioration of the cardiac myocytes, worsening the degree of heart failure, and creating a vicious cycle. Cardiac inefficiency can be calculated by measuring the myocardial oxygen consumption (19). Formatting...

To compensate for the reduced cardiac output, the sympathetic nervous system is upregulated and vagal stimulation of the heart is diminished. The levels of circulating catecholamines and ADH increase and there is activation of the RAAS. These together, bring about tachycardia, increased fluid retention and afterload which in turn elevate the wall stress and the myocardial oxygen demand further (6). The increase in wall tension is compensated for by muscular hypertrophy (19).

Congestive heart failure occurs as the atria push blood into the relatively overfilled ventricles. The cardiac output is decreased, and the heart cannot keep up with the body's demands. Due to ventricular over-filling, the ventricular walls cannot be approximated and complete closure of the atrio-ventricular valves is prevented, resulting in regurgitation (17).

The enlargement of the left ventricle displaces the papillary muscles and causes dilatation of the mitral annulus. The leaflets of the mitral valve retract towards the apex and the mitral valve fails to close, leading to mitral regurgitation (20). The backflow of blood into the atrium along with the poor ventricular function increases left-sided pressure which causes further dilation and remodelling of the left ventricle, decreasing the stroke volume and leading to further deterioration (21).

The conduction pathway in the heart muscle is disturbed by the alteration in the structure of the myocardium, the electrical activity responsible for keeping the heart beating normally is stopped or slowed considerably, resulting in a varying degree of heart block. The "irritable focus" giving rise to the arrhythmia can be a result of myocardial fibre stretching and fibrosis (22). Most of the ion channel dysfunction giving rise to the arrhythmias is within the sarcolemma and occurs due disruption of the sarcolemma-sarcomere link. The ion channel dysfunction can otherwise be due to a gene mutation giving rise to a dysfunctional cytoskeletal protein binding, impairing contractility. 25-30% of DCM patients have LBBB (23). The occurrence of supraventricular arrhythmias should prompt investigation for familial DCM (24). 40% of patients have non-

sustained ventricular arrhythmias – this gives a higher risk of Sudden Cardiac Death [25].

Thrombotic complications are commonly seen in DCM. Cardiac thrombus formation would be expected to be more frequent in the left ventricle, but not exclusively. The underlying cause is the diminished ventricular contractility, wall motion abnormalities and a blood flow disorder within the heart itself, markedly at the left ventricular apex (26). DCM gives rise of heart failure, which is prothrombotic in itself – there is more platelet activation, increased mean platelet volume, higher levels of fibrinogen and D-dimer and reduced levels of ADAMTS-13, which cleaves Von Willebrand Factor (27). Embolisation of the LV thrombi can result in stroke and peripheral emboli while embolisation of right-ventricular thrombi, can result in pulmonary embolism.

Clinical Presentation

The majority present between 20-60 years of age, usually after a lengthy latent period. Eventually, they present with symptoms of LV dysfunction and congestive HF (28). One of the earliest symptoms of DCM is dyspnoea, due to organ under-perfusion and subsequent hypoxia due to pump failure. Orthopnoea may interfere with sleep. This, in combination with the decreased energy production, makes the patient lethargic. Poor exercise tolerance and weakness are non-specific but common symptoms. Syncope and light-headedness can be due to an under-perfused brain, or arrhythmias (6).

Signs of HF can be detected on examination. Jugular vein distension with a hepatojugular reflux, hepatomegaly and pitting oedema of the lower limbs is indicative of right-sided HF (7). Chest auscultation could also reveal pulmonary crackles. A holosystolic murmur would be indicative of mitral and tricuspid regurgitation and an additional S3 gallop would be indicative of blood flowing into the ventricles in diastole (7).

Thrombosis presents according to the affected organ. In stoke, there would be localizing signs depending on the part of the brain affected. In pulmonary embolism there is acute shortness of breath and chest pain. Echocardiography is the most sensitive and specific tool to detect of thrombosis in cardiomyopathies (29).

SCD accounts for 30% of DCM mortality. Although the morality is higher in NYHA functional class IV, due to progressive heart failure, the rates of sudden death is higher in classes I and II. SCD is rarely the initial presentation of DCM. It generally follows the clinical presentation of HF when there is an improvement in the symptoms and is usually due to an arrhythmia (30).

Investigations

Cardiac MRI

Cardiac MRI is the gold standard in identifying both the cause and for monitoring the response to treatment, allowing quantification of ventricular mass, volume and function. Its primary role is differentiation of ischaemic and non-ischaemic cardiomyopathy (31). It also detects thrombi and gives the prognosis of the DCM. Cardiac morphology is assessed by black blood imaging, cardiac function by bright blood imaging, myocardial fat infiltration is assessed by fat saturation

imaging, blood flow is measured by velocity encoding mapping and vascularity by perfusion imaging while vasculature is assessed by gadolinium-enhanced angiography (32).

Echocardiography

An echocardiogram shows the degree of left ventricular dilation, the stage of the disease and the severity of the systolic dysfunction. It also allows measuring of heart size and the visualization of mitral or tricuspid regurgitation, its advantage is that it is not invasive and more easily available (33).

In DCM, LV diameter would be enlarged while the wall thickness would be reduced. The long axis/short axis ratio of the LV, is also reduced as the ventricle adopts a more spherical shape, as seen in Figures 1 and 2. Echocardiography allows calculation of the ejection fraction, which would be reduced to less than 45%. This is due to impaired contractility (34). Mitral regurgitation could be seen by colour flow Doppler echocardiography, as seen in Figure 4. The mitral regurgitation causes the left atrium to dilate, as seen in Figure 4.

3D echocardiography provides a better visualisation of the heart. Tissue Doppler imaging is used to evaluate myocardial diastolic and systolic function, both on a global and regional level. Speckle-tracking echocardiography is a technique used to measure the level of myocardial deformation (35).

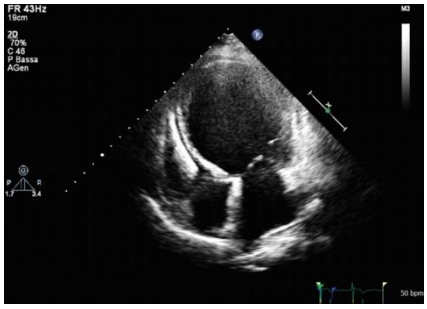

Figure 1: apical 4-chamber view of DCM, showing increased left ventricular sphericity and an implantable defibrillator lead on the right side of the heart. Retrieved from Gil *et al.*, 2016.

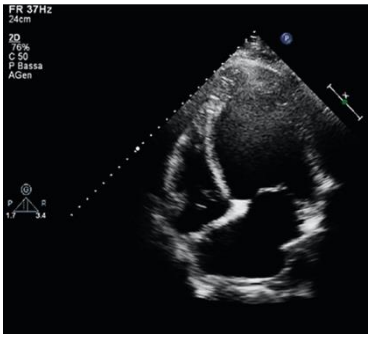

Figure 2: transthoracic echo in apical 4-chamber view, showing extreme remodelling of the heart chambers. Retrieved from Gil et al., 2016.

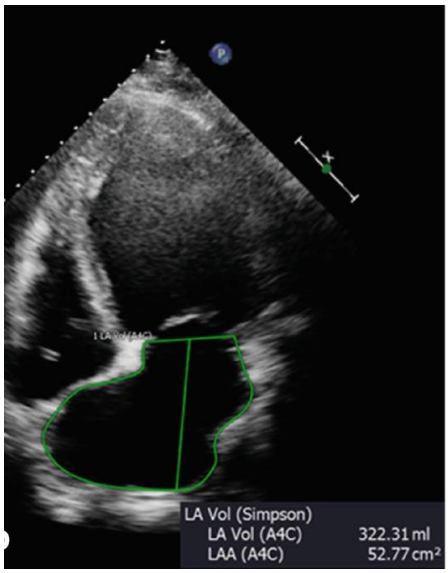

Figure 3: transthoracic echo in apical 4-chamber view, showing severe left atrial enlargement. Retrieved from Gil *et al.,* 2016.

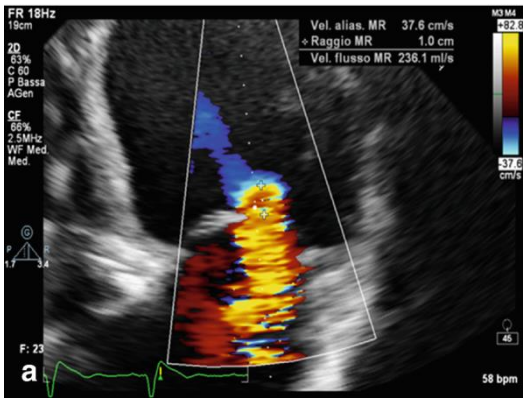

Figure 4: transthoracic echo of DCM with severe mitral regurgitation. Retrieved from Gil et al., 2016.

Plain X-ray

A plain chest x-ray, would show cardiomegaly and in the case of congestive heart failure, signs of pulmonary oedema – batwing hila, Kerley B lines, upper lobe venous distension, increased lung markings, interstitial oedema, and pleural effusion, seen as blunting of the costophrenic angles (36).

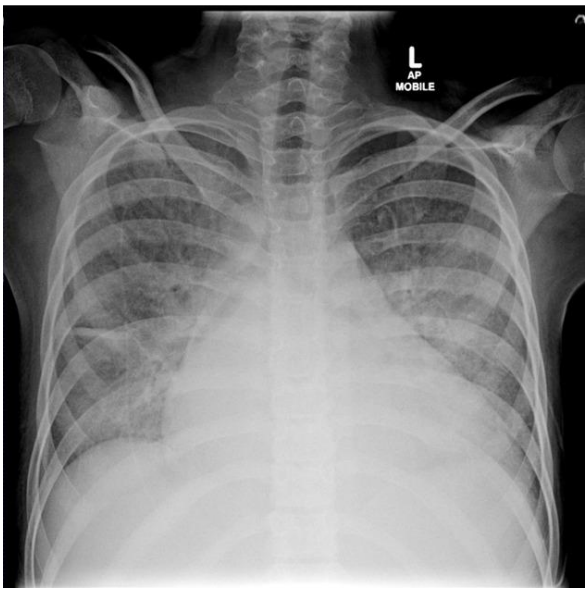

Figure 5: radiographical signs of congestive HF in a young patient, showing cardiomegaly, Kerley B lines, thickening of the interlobar fissures and interstitial oedema. Retrieved from Masarone *et al.*, 2018.

Electrocardiography

Different arrythmias arising from DCM include atrial fibrillation, ventricular ectopic beats, ventricular tachycardia and ventricular fibrillation. Left bundle branch block, may also be seen on the ECG.

Endomyocardial Biopsy

This is an invasive technique which is sometimes used to confirm DCM. It can be useful in cases of iron overload, amyloidosis and other infiltrative diseases but it carries a complication rate of 1-3% (37).

Management and Treatment

Pharmacotherapy aimed at improving the prognosis is required in symptomatic disease, and in asymptomatic left ventricular systolic dysfunction. In both, the onset of heart failure should be prevented by controlling hypertension and increasing the ejection fraction. ACEi, ARBs or ARNI are the preferred drugs to use. Beta-blockers are another possibility (38).

The most obvious and primary intervention would be lifestyle modification to minimize the factors interfering with the cardiac function, such as hypertension and ischaemic heart disease, so maintaining a healthy body weight, cessation of smoking, avoiding

alcohol, reducing sodium intake and restricting fluid intake (39).

Pharmacologic Treatment

The therapeutic algorithm has been proposed by the current European guidelines for patients showing symptoms of heart failure. ACEi (40) are the most effective drugs, replaced by ARBs in case of side effects (41). Beta-blockers are used in concomitance. Incorporation of a mineralocorticoid receptor antagonist would be considered with the dual therapy in persistent heart failure symptoms (42). All three drugs inhibit the sympathetic nervous system, reducing the workload of the heart, hence preserving it. Blocking of the RAAS and the beta-adrenergic system was found to reverse LV remodeling in patients with heart failure and severe LV dilation (19). Several clinical trials conveyed successful outcomes in morbidity and mortality when using the three drugs together.

More recently introduced drugs, ARNI and ivabradine – a hyperpolarization-activated cyclic nucleotide-gated channel blocker, have been added to the recommended list of drugs (39). By inhibiting neprilysin, ARNI inhibits ANP breakdown, hence increasing water loss by the kidney and reducing congestion. Ivabradine is used in patients whose sinus rhythm is over 70bpm at rest whilst on beta-blocker therapy (43). Both drugs were shown to minimize mortality and hospitalization rate of HF patients (44).

Diuretics also decrease symptoms of congestion, but no clinical trials were able to prove its effectiveness in improving mortality and morbidity of heart failure patients (39).

Since dilated cardiomyopathy may give rise to arrythmias, methods of controlling heart rhythm are considered. For ventricular arrythmias, amiodarone is the preferred major anti-arrhythmic agent, especially when the ventricular function is severely depressed. Sotalol is given to treat arrythmias in compensated HF (45).Formatting...

Anticoagulation may be used to prevent the formation of cardiac thrombi, pulmonary and peripheral embolisms [26]. Their use is especially recommended in patients with comorbid atrial fibrillation, history of mural thrombi and artificial valves (28).

Percutaneous Therapies

Catheter ablation can be considered for drug-resistant symptomatic AF and sustained monomorphic ventricular tachycardia (46), whereby heart tissue giving rise to the abnormal electrical activity generating the arrythmia is destroyed via extreme cold or heat. This is carried out by the introduction of catheters through a vein or artery in the groin which are threaded to the heart. This, however, is limited in patients with coronary artery disease.

In chronic heart failure with left ventricular impairment and functional mitral regurgitation, the MitraClip® is used as an alternative option for valve replacement in patients who are surgically high-risk. By preventing the leakage of blood back into the left atrium, it minimises symptoms of heart failure (47).

Device Therapies

An implantable cardioverter-defibrillator is recommended for patient with non-ischaemic DCM, symptomatic heart failure and in instances of a very low ejection fraction. However, it has proven to be more beneficial for patients with ischaemic heart disease than for patients with other HF causes. (39). All candidates for pacemaker implantation should be considered for ICD, even in the absence of LV dysfunction and ventricular tachyarrhythmias (48).

Cardiac resynchronization therapy is recommended by all guidelines for symptomatic HF, especially in non-ischaemic DMC. Despite this, the great majority of patients are non-responders. To minimize the rate of non-response, multimodal cardiac imaging is being used to refine the selection criteria and implantation techniques. Apical rocking seen on apical four-chamber view echocardiogram and myocardial asynchronism seen on cardiac MRI are positive predictors of CRT effectiveness (49).

Operative Treatment

The frequent complication of LV dysfunction in DCM by a regurgitant mitral valve necessitates its treatment. Mitral valve repair by undersized ring annuloplasty is a widely used option that leads to reversal of LV remodeling, and improves morbidity. It improves mortality in patients with moderate to severe mitral regurgitation secondary to idiopathic or ischemic DCM. However, it is limited by residual and recurrent mitral regurgitation in functional mitral regurgitation. To prevent recurrent or persistent mitral insufficiency, mitral valve replacement is preferred, especially in the presence of predictors of repair failure in the pre-operative echocardiogram (50).

Patients whose conditions are not improved by medical therapy develop chronic heart failure. For these patients, mechanical circulatory support (MCS) devices are necessary to unload the failing ventricle and improve organ perfusion. Short-term MCSs stabilise the haemodynamics and help recovery of patients with acute heart failure or cardiogenic shock until a more permanent solution is decided (45).

A left-ventricular assist device (LVAD) may be used to assist the left ventricle of the heart in pumping blood to the rest of the body. It is a battery-operated mechanical pump used in patients at end-stage heart failure. The LVAD has proved to be a successful alternative to a heart transplant, which is limited by donor availability. A heart transplant increases greatly the quality of life of patients with chronic heart failure who have no alternative choices (45).

Preventing Sudden Cardiac Death

Cardiac MRI and Holter monitoring are used to monitor risk stratification of HF progression and SCD (51). In LV dysfunction, with an EF of 35% of less, ICD reduces SCD by 80%. As a result, ICD is also being considered in familial cardiomyopathy with SCD, impaired LV function without HF symptoms and DCM with sustained ventricular tachycardia or syncope (52). Prior to consideration of ICD therapy, the HF has to be optimally controlled for 6 months and reassessment of LV function recovery has to be carried out beforehand, unless there is a significant risk such as familial DCM with ventricular arrythmias (30).

Familial Screening

Echocardiography and electrocardiogram should be used to screen all first-degree family members of affected patients. extensive family members should only be considered for testing if they have a high-risk occupation. Genetic testing is carried out depending on the clinical features of the presenting disease. Genetic testing offers an opportunity for early identification and intervention to stop or reverse the disease progression (5). Although seemingly beneficial, screening has its repercussions as well – detection of familial DCM with reduced penetrance, meaning that the individuals will not exhibit the signs of disease would mean unnecessary worry and treatment. To prevent this, only the clinically affected family members should receive pharmacological treatment based on their symptoms and their severity while a follow-up cardiac screening approach should be adopted for asymptomatic relatives, with ECG and echocardiography to detect even minor changes (53).

A Multidisciplinary Approach

This approach needs to be taken both to determine the cause of the disease and because several systems may be affected, either by the complications of the disease itself or else by the primary cause. In addition to the cardiac electrophysiologists, heart failure specialists and radiologists, which you would expect to be involved in all cases of DCM, the involvement of other specialist physicians would be specific to each case. For instance, DCM arising from sarcoidosis would most likely require the input from a respiratory physician as the lung is more commonly affected than the heart, in autoimmune disease, rheumatologist input would be required, neurologist involvement in neuromuscular disease, hematologists in DCM arising from iron overload and oncologists when arising from cytotoxic complications of chemotherapy (54). The management of the outcomes of the disease also calls for a multidisciplinary approach. In familial DCM, there is geneticist involvement to tackle the risk of relatives, as well as determining the type, risk of SCD and possibly guiding the management. Considering that congestive heart failure increases the risk for kidney disease, owing to congestion and hypoxia from hypoperfusion, which impairs urine output and exacerbates the HF, nephrologist involvement might be beneficial (55). If the patient is an athlete, consultation with a sports physician would be ideal.

Introducing the patient to a heart failure clinic which personalizes a management plan encourages the patient to have an active role in managing the disease. This programme would involve nurses, physicians and the appropriate specialist input as well as the patients themselves. Heart failure clinics educate the patient on how to adequately manage the disease through appropriate lifestyle modifications, exercise and medication while offering regular follow-up and close monitoring of the disease progression and response to treatment.

Conclusion

In essence, the diagnosis of DCM can be easily made by the use of appropriate investigations, most notably the cardiac MRI and the echo. The management is broad, and highly specific to each case but essentially, its aim is to optimise the cardiovascular health and to minimise symptoms and complications

of the disease, one of the most important being heart failure. Despite the multiple forms of treatment and prophylactic approaches previously mentioned, many patients still develop heart failure and as much as 50% die within 5 years (28).

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder, involving a progressive degeneration of upper and lower motor neurons, resulting in loss of motor function and eventual death. This scientific review will tackle the disease from multiple aspects so to shed light on both the clinical features and pathophysiological mechanisms of the disease. The clinical aspect will emphasize the different clinical phenotypes and touches upon the clinical overlap of frontotemporal dementia and ALS. The pathophysiology will focus on the genetic factors, contrasting the genetic architecture found in European ALS patients with that of the Maltese patients. The major pathological mechanisms involved in ALS will be mentioned. Additionally, the known environmental factors of ALS will be reviewed. Lastly, the therapeutic approaches will be discussed. In this section, riluzole is discussed, but emphasis is placed on the evolving, highly efficacious RNA-based therapy.

Introduction

Amyotrophic lateral sclerosis (ALS) is a chronic neurodegenerative disorder, characterized by a progressive degeneration of motor neurons, typically involving both upper and lower motor neurons, resulting in weakened motor function. Disease onset involves muscle weakness at a focal point and spreads immediately to affect numerous muscles. Therefore, the independence of the patient suddenly diminishes and death usually occurs after only 3-5 years, due to respiratory failure (following paralysis of the diaphragm), but survival varies from one individual to another (1).

Traditionally, ALS is classified into sporadic and familial. In familial ALS (fALS) there is history of ALS in a first- or second-degree relative and inheritance generally, but not exclusively, involves dominant traits (1). The remaining ALS patients are classified as sporadic, i.e., disease with no notable family history (2). Nevertheless, this does not mean that genes do not have a role in the development of sporadic ALS. In fact, around 10% of sALS cases show gene mutations that are common to those associated with fALS and the risk of first-degree relatives to suffer from ALS increases eight-fold, even with sALS (3,4).

ALS is the most frequent motor neuron disease (MND) in adults, with a median

prevalence of 5.40 per 100,000 population and an annual incidence rate of about 2.08 per 100,000 population, in European and European-descent populations (5). Both parameters increase with age; however, the risk of developing ALS rises until 50-75 years, whereby the risk then starts to decline. As a matter of fact, onset of disease occurs around the age of 65 years for sALS (3). Onset of ALS in early adulthood may indicate fALS (1). The lifetime risk for developing ALS is higher in males than in females, by a factor of 1.2 – 1.5 (6).

ALS incidence and prevalence in Malta follow the European statistics quite similarly, with a prevalence of 3.44 per 100,000 (as of 31st December 2018) and an annual incidence rate of 2.48 per 100,000 population (studied over a 2-year period, 2017-2018). The median onset of disease is 64 years for males and 59.5 years for females, as opposed to northern populations of the Mediterranean, where the age at onset of disease is higher in females. Males are also at a greater risk of developing ALS in Malta, across all age groups above the age of 49 (Fig. 1). As aforementioned, the risk of ALS increases with age. In Malta this is also the case up till the age of 79 years, from which the incidence decreases, for both males and females. As seen in figure 1, there are differences in the incidence peaks between males and females. The peak for males occurs in the 50-59 age group, whereas the peak in females occurs in the 70-79 age group (7).

A noteworthy remark of the Maltese population is that quite a significant number of cases, 12.5%, involve fALS (7). This number is relatively high when considering the 5% rate reported from the meta-analysis of various population-based registries (8). However, the frequency of fALS in the Maltese frequency is similar to that found in Liguria (northern Italy), which is 10% (9).

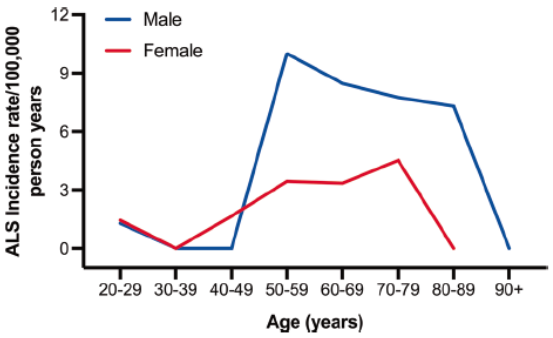

Figure 2: The ALS incidence rate in Malta with advancing age. Males (blue line) are at a higher risk of developing ALS than females, after the age of 49. The incidence of ALS increases with advancing age, until the age of 79, peaking at 50-59 years in males (blue) and 70-79 years in females (red).

Clinical Aspects of ALS

Symptoms and Clinical Phenotypes

ALS is considered clinically complex as there are many possible disease outcomes – a characteristic known as heterogeneity. Classification of clinical phenotypes can be done according to the motor neuron involved, i.e., upper motor neuron (UMN) and/or lower motor neuron (LMN), or according to the disease onset.

Degeneration of UMN presents with spasticity and weakness of muscles and the important clinical finding of hyperreflexia. Whereas degeneration of LMN leads to muscle weakness, atrophy, fasciculations and hyporeflexia/areflexia (10). Due to these pathological changes, patients with ALS often complain of fatigue and a reduced ability to carry out physical activity (11). Many ALS patients also present with clinically severe

weight loss. The cause of the weight loss is often related to the motor deficit in bulbar muscles, which leads to dysphagia. However, spinal-onset ALS also presents with weight loss and this shows that other factors such as hypermetabolism, reduced appetite and cachexia, that accompany ALS, also contribute to weight loss (12). Weight loss is important to tackle in management since reduced body mass index shows poorer prognosis (13).

Several motor neurons are affected in ALS, and this accounts for the heterogenous presentations. However, the oculomotor nuclei and Onuf's nucleus are spared and this is why eye movement and sphincters remain intact, until late in the disease (3).

The disease signs are initially exhibited at focal random regions of the body, such as masticatory muscles (bulbar) or thigh muscles. It is in this area where there is maximal degeneration of the UMN and LMN. Then, the symptoms at the same focal region worsen with time and they start to spread to contiguous areas of the body, not only to local regions (e.g., from arm to forearm), but also to neuroanatomically-linked regions (e.g., to contralateral side) (14). Since UMN and LMN have different somatotopic organization and spread distances, UMN and LMN deficits may appear discordant to each other, further adding to the complexity of the clinical presentation of ALS (15).

Apart from motor deficit, ALS commonly affects non-motor regions. Around half of ALS patients show cognition/behavioural impairment sometime during disease (14). A common manifestation of non-motor involvement is frontotemporal dementia, seen in 13% of incident cases (16). Other less common features of ALS, mostly associated with advanced disease, are extrapyramidal effects, supranuclear gaze palsy and autonomic nervous system involvement (10).

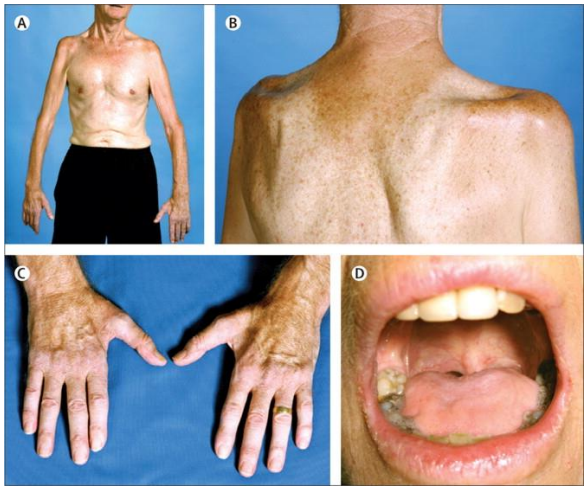

Figure 3: Clinical presentations of muscle atrophy in ALS. A: flail arm/man-in-a-barrel syndrome, with wasting of the proximal upper extremity muscles. **B**: muscle atrophy of the shoulder muscles. **C**: unilateral atrophy of the thenar muscles. **D**: atrophy of the tongue muscles and an absent elevation of the soft palate on vocalisation. Retrieved from Kiernan *et al.*, 2011.

Overlap of frontotemporal dementia and ALS

Recently, there has been much more research into frontotemporal dementia (FTD) and its overlap with ALS, both at the clinical and neuropathological level (17). Nowadays, it is believed that ALS and FTD are the two extremes of a spectrum of one disease, that is known as the MND-FTD continuum (Fig. 3) (3).

FTD, like ALS, is a progressive neurodegenerative disease that initiates at a focal point and eventually results in atrophy of the frontal and temporal lobes of the brain, due to the substantial loss of neurons (14).

The condition has two main subtypes: the behavioural variant and the primary progressive aphasias (which are further divided). FTD involves an immediate and devastating loss of independence as the individual starts showing progressive behavioural and cognitive decline, involving emotional instability, executive dysfunction and language deficit (18). Symptoms initiate at a relatively young age, most often in mid-life, and it is considered the second most common form of early-onset dementia under the age of 65 (17).

In conjunction with ALS, about 5-15% of patients have FTD, which is known as ALS-FTD. Whereas, up to half of ALS patients do not satisfy the diagnostic criteria for FTD, but still show behavioural or cognitive changes. This latter group of ALS patients can present with either behavioural impairment, executive dysfunction, or non-executive dysfunction, in addition to the ALS symptoms. Moreover, there are some individuals who are primarily diagnosed with FTD but develop motor neuron involvement later on and are known as FTD-MND (3).

It is the behavioural variant of FTD that mostly affects ALS patients. In fact, 10% of all ALS patients show behavioural symptoms. Most of such symptoms include apathy and loss of sympathy. When cognition is affected, aphasia, impaired social cognition, and executive dysregulation, are the most common symptoms encountered. Memory involvement is not a common occurrence (3).

Apart from the clinical overlap, ALS and FTD are linked at the molecular level. Many of the patients suffering from either ALS or FTD show TDP43 proteinopathy as a common feature. However, the hexanucleotide repeat expansion (HRE) mutation in the *C9orf72* gene, denoted as *C9orf72*$^{(GGGGCC)exp}$, was the discovery that truly linked ALS and FTD on a spectrum (10).

Survival is significantly affected when FTD signs accompany ALS. In fact, ALS-FTD patients have a survival of only 2.4 years from onset of disease, which is a year less than what is expected in typical ALS. Other than the poor prognosis, FTD often leads to the patient not complying to the care being undertaken and this puts a further strain on the healthcare workers and the patient's relatives. Cognitive decline will also interfere with the autonomy of the patient, and thus, may involve the need of medico-legal assistance due to ethical issues that may ensue (3).

The Pathogenesis of ALS

Genetic factors

Understanding the genetic aetiology of ALS is fundamental since it helps reveal the pathophysiological processes underlying the disease, which would allow for the development of more targeted therapy.

Mendelian inheritance is exhibited by some ALS genes, mostly in an autosomal dominant manner, characterized by high penetrance (19). Nonetheless, sALS does not show any family history and thus, this condition is far more complex genetically. Unlike many genetic disorders that depend on the presence of a large number of common genetic variants, studies on seemingly sporadic ALS cases show that the disease is mainly based on rare genetic variants that may be specific to families. These two features make the identification of

these rare variants more advanced and complicated (16).

Over 40 genes have been discovered to be associated with ALS thus far (Fig. 4). The first ALS gene to be discovered was the *SOD1* gene. There were a relatively few gene discoveries up until 2004, from which discoveries became much more frequent (Fig. 4) due to technological advances (20). Nowadays, the genetic factors accounting for two thirds of fALS cases and 10% of sALS cases are known (21).

Genetic factors involved generally exhibit missense substitutions, whereby there is a single nucleotide variant (SNV) that results in a different amino acid in the product. However, the *C9orf72* gene shows a large expansion of the GGGGCC hexanucleotide repeat in the first intron and not a missense substitution. weakness. Whereas, when there are 27-33 CAG repeats, the susceptibility of developing ALS is increased (19).

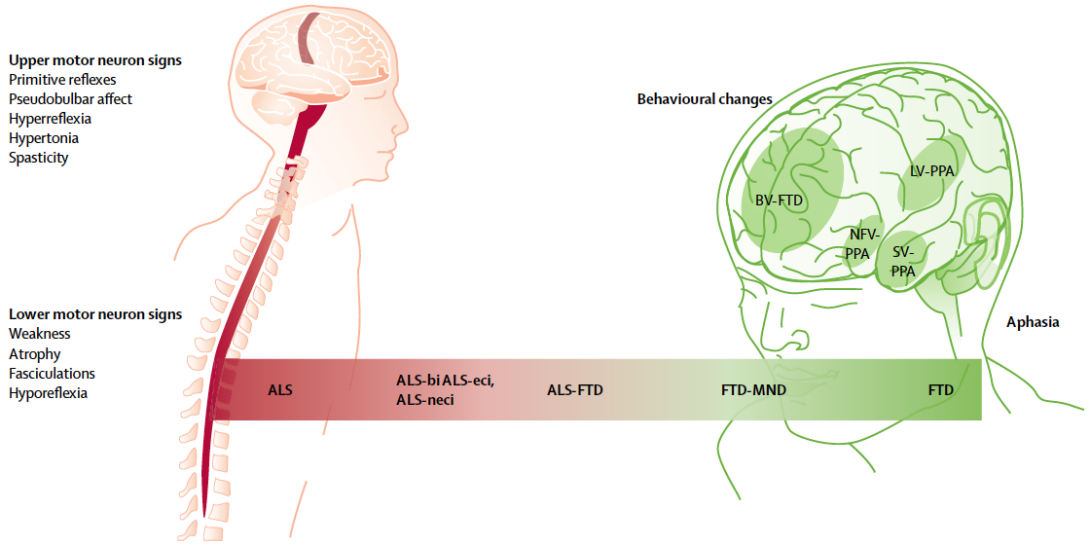

Figure 4: ALS and FTD as extremes on a spectrum & the clinical phenotypes in between. ALS-bi denotes behavioural changes, ALS-eci denotes executive dysfunction and ALS-neci denotes non-executive dysfunction with other forms of cognitive impairment (such as memory impairment). Retrieved from van Es Ma *et al.*, 2017.

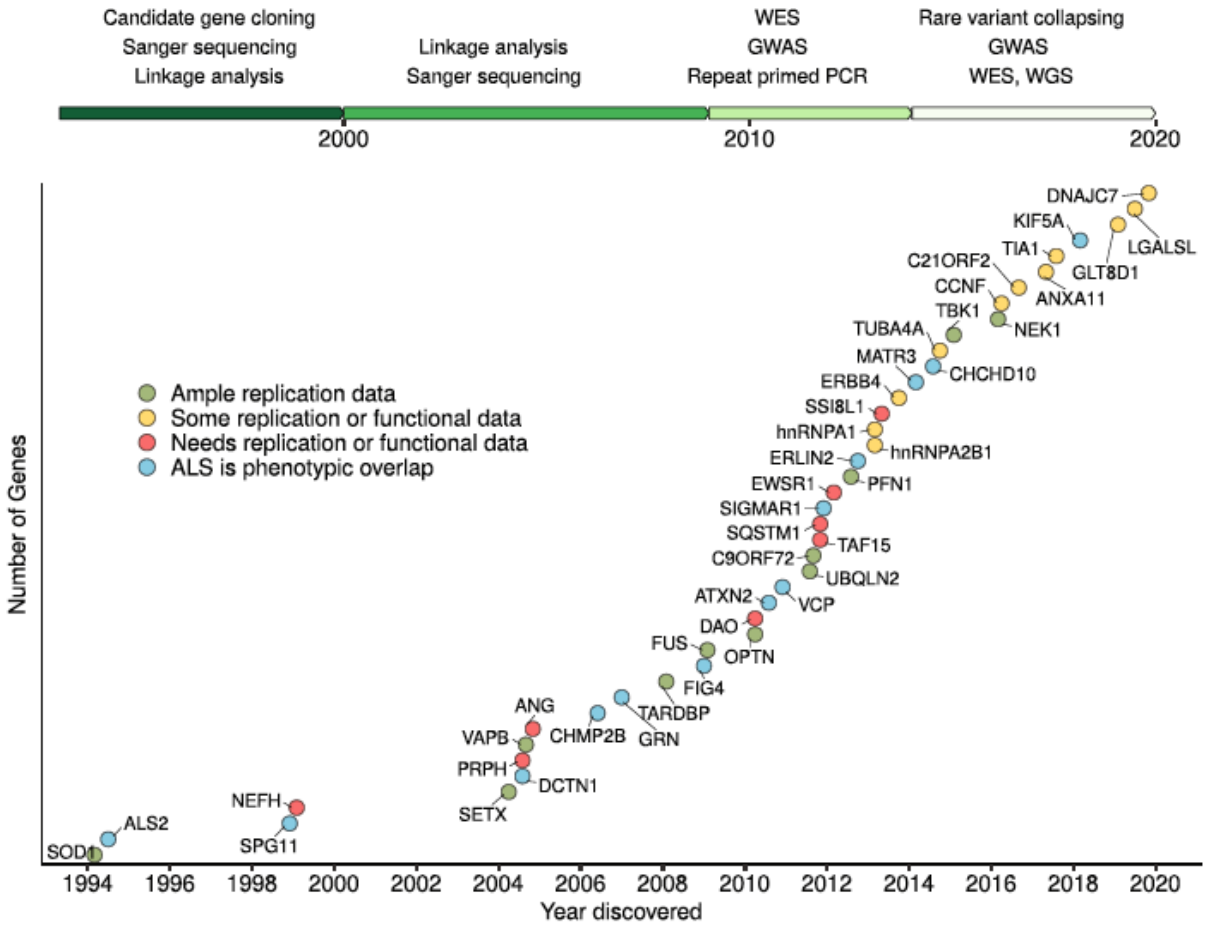

Figure 5: ALS gene discovery and its evolution. From the first gene discovery in 1994, there was a lag due to lack of advanced and efficient gene sequencing technology. This graph is showing 43 ALS genes that were discovered. Abbreviations: GWAS: genome-wide association studies; WES: whole-exome sequencing; WGS: whole-genome sequencing. Retrieved from Belbasis *et al.*, 2016.

The effects that such gene variations have on ALS are varied and include inducing motor neuron degeneration, increasing patient susceptibility and/or affecting the rate of progression. A typical example involves the ATXN2 gene. An expansion of the CAG trinucleotide repeat in the coding sequence of the ATXN2 can have two outcomes, depending on the number of repeats involved in the expansion. When there are more than *34* CAG repeats, spinocerebellar ataxia type 2 is caused, which can mimic ALS as it may present with motor weakness. Whereas, when there are 27-33 CAG repeats, the susceptibility of developing ALS is increased (19).

The major gene variants exhibited in ALS involve the *SOD1*, *TARDBP*, *C9orf72* and *FUS* genes. Their frequency differs from one population to the other, however, in the European population, the *C9orf72* gene is most commonly involved in ALS, followed by *SOD1*, *TARDBP* and *FUS* gene involvement, as seen in figure 5 (22).

While variation in the above four genes is most seen in ALS, geographic isolation can result in an altered frequency due to natural

consequences observed in population genetics, such as the Founder effect.

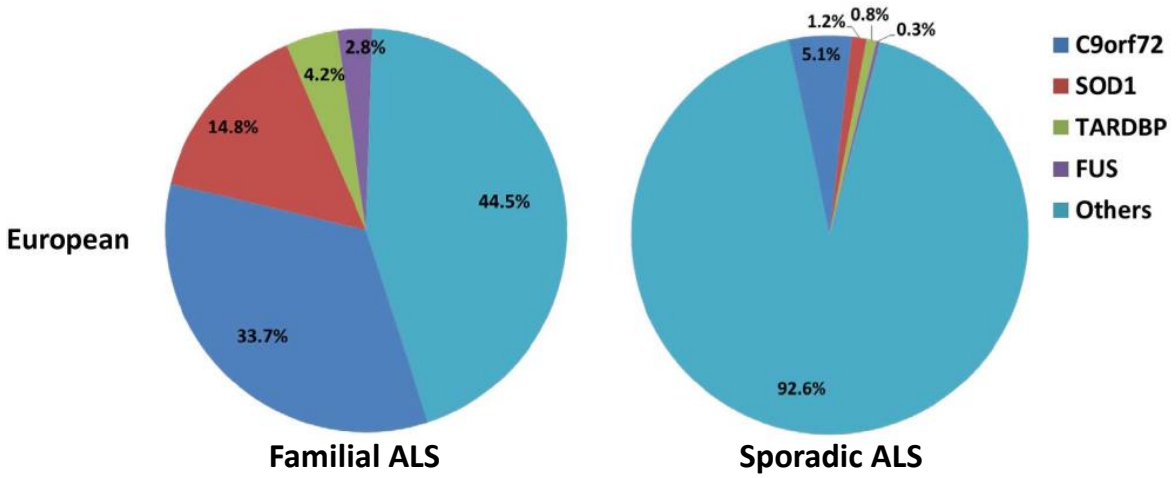

Figure 6: The genetic architecture of familial and sporadic ALS in the European population. The two pie charts are depicting the frequency of ALS cases according to the gene involved. In both fALS and sALS, the main contributing genes, sorted from most common to least common, are *C9orf72*, *SOD1*, *TARDBP* and *FUS* genes *(22)*.

This is the case in Malta, whereby, a recent study revealed that the damaging variants in *C9orf72*, *SOD1*, *TARDBP* and *FUS* genes were absent in the Maltese ALS patients. Instead, the genes *ALS2*, *SCFD1*, *DCTN1*, *SPG11*, *ERBB4*, *DAO*, *NIPA1*, *ATXN2* and *SETX*, which are considered as 'minor', were involved in ALS. This discovery has highly significant implications as it means that advances in therapy targeting the European population may not be suitable for the Maltese patients (7).

Environmental and lifestyle factors

A considerable portion of sALS cases cannot be explained by genetic factors and these are attributed to environmental factors and/or to yet-unidentified factors (1).

While genetic knowledge regarding ALS is developing at a relatively fast pace due to the availability of more-advanced technology, environmental factors cannot be clearly studied due to several reasons, e.g., it is difficult and may be inaccurate to recall all the exposures in one's life.

Regardless, there are still some environmental and lifestyle factors that are suspected to be associated with ALS. A highly probable environmental/lifestyle factor is cigarette smoking, which may account for earlier onset of the disease. An evident decrease in ALS risk is observed upon increasing the time-since-quitting (in years), in fact, the risk is halved within 5 years of quitting. There are many proposed mechanisms stating why cigarette smoking increases ALS risk, including the exposure to oxidizing chemicals, lead and formaldehyde (23).

Physical activity is a debated lifestyle factor, however a large study confirmed that there is an independent linear relationship between physical activity and ALS risk. While

strenuous physical activity carried out by professional athletes increases the risk substantially, recreational physical activity, of moderate intensity, also increases the risk (24). This risk explains why ALS is referred to as 'Lou Gehrig's disease' in the United States. Lou Gehrig was a professional baseball player who developed ALS in his thirties, and this sparked an interest in the possible association between professional athleticism and ALS. An emphasis is now being placed on those sports that involve repetitive head trauma, due to the risk of developing chronic traumatic encephalopathy. The latter results in frontotemporal atrophy and deposition of tau protein (characteristic of Alzheimer's and Parkinson's diseases) and of TDP43 protein (characteristic of ALS) (1).

Military service is another probable risk factor of ALS. While certain studies do not attest, one study that is of a higher quality revealed that veterans show an ALS risk that is 1.3 times greater than that of their civilian counterparts. This increase in risk may be due to involvement of vigorous physical activity, trauma and exposure to smoke, lead and pesticides (25).

Lead is also associated with increased ALS risk. It is one of the few environmental factors that is greatly supported in its causal link to ALS, via several studies (26–28). An interesting study, was a questionnaire-based case-control study, which revealed that even participating in hobbies that involve lead is associated with an increased ALS risk (29). The pathophysiology behind this environmental factor is still unclear, however it is known that lead increases free radical formation, causes perioxidative damage to cell walls, leads to aggregation of insoluble TDP43 and induces neuronal cell death (30).

Air pollution was also observed to increase the risk of ALS. A population-based case-control study conducted in the Netherlands revealed that traffic-related air pollutants, specifically $PM_{2.5}$ absorbance and the nitrogen oxides, contributed to the increased ALS risk (31). An important source of traffic-related air pollution is diesel exhaust and its association with ALS has been revealed in various occupational studies. Another study in 2015, found a potential association between ambient air pollution, specifically aromatic solvents, and ALS (32).

Lastly, pesticides are also considered an environmental risk factor of ALS. Indeed, persons working within the agricultural sector who are exposed to agricultural chemicals (mostly pesticides) long-term were at an increased risk of developing ALS. This was especially true when the work duration amounted to or exceeded 10 years. From the pesticides, fungicides were found to have a higher association with ALS. Of note, pesticide usage in homes was not associated with an increased ALS risk (33).

The pathology of ALS

ALS involves the degeneration of motor neurons at multiple sites of the nervous system: the motor cortex of the brain (UMN), the corticospinal tract (UMN), the motor nuclei in the brainstem (LMN) and the anterior horn cells in the spinal cord (LMN). Degeneration of the UMN in the lateral corticospinal tract leads to scarring (19). While LMN degeneration results in skeletal muscle atrophy and features of denervation and reinnervation (16).

As neuron degeneration progresses, molecular neuropathology develops. This involves the presence of dense and round, or skein-like protein aggregates, known as inclusions, in the cytoplasm of the motor neurons (14). These cytoplasmic inclusions are often ubiquitinated and the hallmark feature of ALS neuropathology is the deposition of ubiquitinated TDP43 protein, which is encoded by *TARDBP* gene. This is a recurring feature, seen in most ALS cases (both familial and sporadic), except those cases in which the *SOD1* and the *FUS* genes are mutated as these show SOD1 and FUS protein inclusions, respectively. Thus, TDP43 function is considered important in the pathogenesis of ALS (3). Those ALS cases involving the *C9orf72* gene variant exhibit an atypical combination of inclusions consisting of abundant p62 positive, TDP43 negative ubiquitinated intranuclear inclusions, especially found in the cerebellum and hippocampus (14).

Apart from these molecular findings in motor neurons, non-neural cells also show pathology. This is manifested as astrogliosis, spongiosis and microgliosis. It is a combination of neural and non-neural cell pathology that results in ALS (19).

The cellular pathogenic mechanisms of ALS

In this review, focus is made on five major mechanisms involved in ALS, namely protein homeostasis, ribonucleic acid (RNA) metabolism, axonal transport, glutamate-mediated excitotoxicity, and mitochondrial dysfunction.

Aberrant protein homeostasis

Considering that protein aggregates (inclusions) are a classic pathological feature of ALS, protein homeostasis is a major mechanism in the pathogenesis of ALS. The basis of this mechanism lies in the nature of the translated product of the damaging gene variant, wherein the protein produced can be misfolded, mislocalised, or aberrant (16). In this abnormal form, it has a number of downstream effects, including a direct effect on autophagy and the ubiquitin-proteasome system (UPS), the two major pathways by which proteins are cleared in the cell (19).

When these protein clearance pathways are disrupted, there is an accumulation of various proteins which has toxic effects. Mutated TDP43, SOD1 and FUS proteins are the three major proteins to misfold and form aggregates. These protein aggregates become prion-like via self-assembly and propagation to other cells, resembling how ALS initiates at a focal point and then spreads to other areas. This prion-like spread has become a hallmark feature in many neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease (15,19,34).

Impaired RNA metabolism

Like in all cells, messenger RNA (mRNA) is produced in the nucleus and is transported to the cytoplasm, where translation occurs. However, in neurons, once the mRNA is in the cytoplasm it can be transported to distal parts, into the axonal compartment (16).

The TDP43, FUS and hnRNP A1 proteins all form part of the heterogenous nuclear ribonucleoproteins (hnRNP) family and when

mutated, they leave the nucleus and are mislocalised in the cytoplasm. In this manner, the proteins can no longer bind to RNA, and this has drastic effects on RNA processing, since one RNA-binding protein normally binds to many RNA targets. Thus, the normal function of the nucleus is significantly impaired, e.g., mutant TDP43 protein disrupts alternative splicing (19,35).

The *C9orf72* gene greatly contributes to this mechanism. The HREs are key in causing disease and this is done via multiple pathways, involving both loss of function and gain of function (Fig. 6B) (36).

Additionally, the generation of microRNAs (miRNAs) is reduced when there are damaging variants affecting TDP43 and FUS proteins. miRNA are non-coding RNAs that have a role in silencing gene expression and maintaining neuromuscular junctions (19). Due to the reduced levels of miRNAs in ALS patients, they are considered as promising biomarkers, which could help improve the patients' quality of life though more-targeted therapy (37).

Altered axonal cytoskeletal dynamics

The axonal compartment of the neuron depends on the cell body for biosynthesis and therefore, components are shuttled between the cell body and the terminal end of the axon via the cytoskeleton. There are two modes of transport: anterograde (from cell body to axon terminal) and retrograde (from axon terminal to cell body). The cytoskeleton is comprised of microtubules, and they form a network onto which motor proteins, e.g., dynein, move.

The proteins dynactin subunit 1 (encoded by *DCTN1*), profilin-1 (encoded by *PFN1*) and tubulin α-4A chain (encoded by *TUBA4A*) are essential in maintaining cytoskeletal dynamics. Therefore, damaging variants in these genes, as can be seen in ALS, has a significant impact on axonal transport.

In addition, *SOD1* variation is known to slow down both anterograde (38) and retrograde (39,40) transport before neurodegeneration initiates. Apart from *SOD1*, those genes encoding the RNA-binding proteins have a contribution in this transport, since RNA processing in the axonal compartment requires the delivery of the required components (e.g., ribosomes) from the cell body (19).

There also exists a relationship between *EPHA4* expression and axonal extension, wherein reduced expression of *EPHA4* is associated with greater axonal extension (1). Finally, the *NEFH* and *PRPH* genes, which are involved in the maintenance of neurofilaments, are also implicated in ALS, but they are seen in a smaller number of cases (16,41,42).

Glutamate-mediated excitotoxicity

This is one of the oldest mechanisms to be proposed in the pathophysiology of the neurodegeneration involved in ALS (43). This phenomenon is characterized by excess glutamate (Glu) neurotransmitter in the synaptic cleft, resulting in a prolonged activation of Glu receptors, which causes neurotoxicity. Glu-mediated excitotoxicity is implicated in other diseases, including Alzheimer's disease (44).

There are various Glu receptors. The inotropic Glu receptors (iGluR) are predominantly involved in the Glu-mediated excitotoxicity, with the NMDA receptor (NMDAR), a subtype of iGluR, being the key player (44).

Calcium plays a pivotal role in the molecular mechanism of Glu-mediated neurotoxicity – hence, why NMDAR is the key receptor due to its high calcium permeability (45). Prolonged activation of NMDAR results in a drastic increase in intracellular calcium concentration and this has many downstream effects that are cytotoxic. To decrease the effects of the drastic increase in calcium, the mitochondria capture some of the calcium (46). Being a cation, excessive uptake of calcium results in depolarization of the mitochondrial membrane and this has adverse effects on the production of ATP, leading to a low-energy state, with an abundance of ROS (45). Nonetheless, the pro-oxidant enzyme nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase is the greatest contributor to reactive oxygen species (ROS) production following excessive Glu exposure (47).

Astrocytes are intrinsically involved in this process as they regulate the Glu levels in the synaptic cleft by maintaining its synthesis and clearing and recycling it via the glutamate-glutamine cycle. Glu uptake by the astrocyte is mediated by the excitatory amino acid transporter 2 (EAAT2), which transports Glu from the synaptic cleft to the astrocyte and is sodium-dependent. In ALS, there is astrocyte-mediated downregulation in the production of EAAT2, encoded by *SLC1A2*, thereby resulting in less Glu uptake (44). It has recently become known that membralin, a protein residing in the endoplasmic reticulum membrane, also induces EAAT2 downregulation, via the TNF-α/TNF receptor 1/nuclear factor κB pathway (48). Apart from this, ALS causes astrocytes to release neurotoxic factors, which exacerbate neurodegeneration. Also, astrocytes release less neurotrophic factors, which normally function in neuronal growth and development (44).

Mitochondrial dysfunction

Mitochondria are of great importance to neurons which have relatively high metabolic requirements.

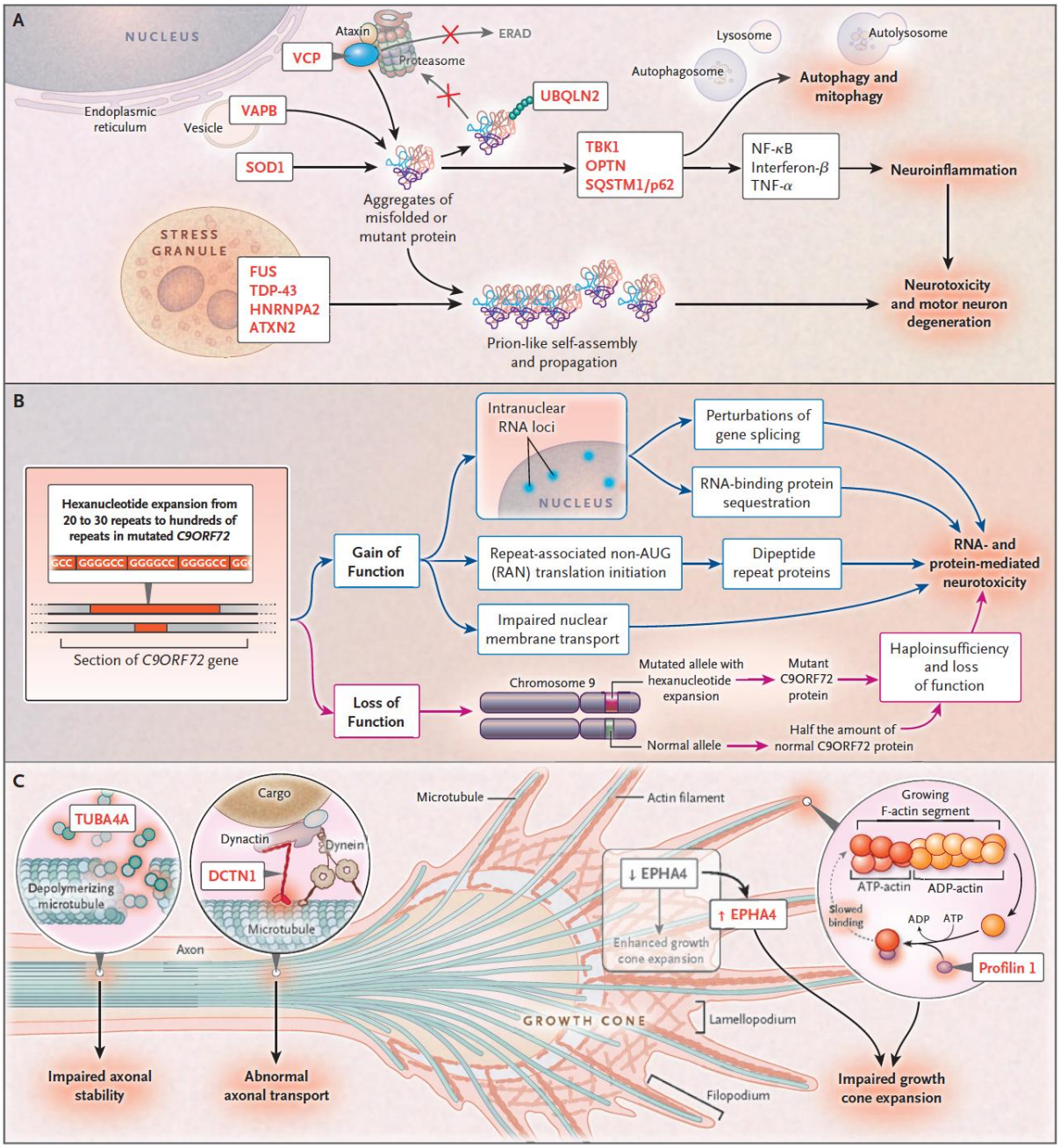

Figure 7: Protein homeostasis, RNA metabolism and axonal cytoskeletal dynamics, and their implications in ALS. Panel A concerns altered protein homeostasis, through disruption of protein clearance via endoplasmic reticulum-associated degradation (ERAD), the ubiquitin-proteasome system and autophagy. Moreover, there is the prion-like spread of misfolded protein. Panel B concerns disrupted RNA metabolism, specifically the mechanisms of the *C9orf72* gene variant. The expansion of the intronic hexanucleotide repeat has several gain-of-function effects, including the formation of RNA foci, formation of dipeptide repeat proteins and altered nucleocytoplasmic transport, all of which are toxic to the neuron. In addition, there can be loss of function, where the decreased levels of C9orf72 protein are toxic to the neuron. Panel C concerns impaired cytoskeletal dynamics, particularly the effects of gene variants in *TUBA4A*, *DCTN1*, *EPHA4* and *PFN1* (profilin 1). There is disruption of axonal transport, cytoskeletal integrity and axonal expansion. Retrieved from Brown *et al.*, 2017.

These organelles are important not only as producers of ATP via oxidative phosphorylation, but also for their role in apoptosis and as calcium buffering organelles (49,50). One must keep in mind that neurons are post-mitotic (i.e., long-lived cells) and thus, they are more vulnerable to the cumulative damage of mitochondrial dysfunction (50,51). There are many facets to ALS-associated mitochondrial dysfunction, including, impaired oxidative phosphorylation, defective calcium homeostasis, activation of apoptosis and oxidative stress via production of ROS.

In the past, mitochondrial dysfunction in ALS was mostly linked with *SOD1* mutations – however, evidence has been emerging that other familial ALS causes, particularly mutations in *TARDBP* and *C9orf72* also contribute to both functional and morphological defects in mitochondria (36).

An integral part of inducing mitochondrial damage is the direct interaction of ALS-associated proteins with the mitochondria itself. For example, mutant SOD1 protein interferes with mitochondrial function by localising to the intermembrane space, where it aggregates and reduces the activity of the electron transport chain (ETC). Additionally, this mutant protein interferes with the exchange of ATP, ADP, and others across the outer mitochondrial membrane via disruption of voltage-dependent anion channel 1 (VDAC1). TDP43 disrupts mitochondrial function via a different mechanism: it targets mRNA and impairs the transcription of components of complex I of the ETC. Furthermore, ALS mutant FUS protein is associated with increased ROS production and reduced ATP production (49). In a recent study, it was shown that C9orf72 has a role as a mitochondrial-inner-membrane-associated protein that stabilises a component of complex I of the electron transport chain and thus, helps regulate oxidative phosphorylation. Therefore, haploinsufficiency and loss of function of *C9orf72* in ALS resulted in a reduction in complex I activity (52). Overall, in all cases there is a trend of decreased ETC activity, decreased ATP production and increased oxidative stress. Considering the high energy requirements of neurons, ATP depletion may thus, trigger neuron degeneration (49).

Mitochondrial dysfunction in ALS also involves apoptosis, an important mechanism by which a damaged cell is removed from the environment in a controlled manner. The mechanism is regulated by the mitochondria via pro-apoptotic and anti-apoptotic proteins of the Bcl-2 family. Pro-apoptotic signalling in ALS is mostly an indirect consequence of other toxic events. However, it was discovered that mutant SOD1 protein directly affects apoptotic signalling by sequestering the anti-apoptotic protein Bcl-2, resulting in a pro-apoptotic state (53).

Mitochondria have the essential role of buffering surges of calcium (Ca^{2+}) within excited cells and hence, these organelles are particularly important in excitable cells such as motor neurons. Ca^{2+} buffering and uptake into the mitochondria is a finely tuned event that is of great significance not just for normal mitochondrial function, but for overall cellular homeostasis (36,50). Of note, Ca^{2+} uptake depends on the mitochondrial membrane potential, which in turn depends on mitochondrial respiration via the electron transport chain (36).

It has recently emerged that Ca^{2+} miscommunication between the endoplasmic

reticulum (ER) and mitochondria is a major contributor to calcium mishandling in ALS. Normally, the membranes of the ER and mitochondria are closely connected via several protein complexes on the outer mitochondrial membrane. It was discovered that these complexes were disrupted in SOD-1, TDP43 and FUS-related ALS. Specifically, in the TDP43 and FUS models, this miscommunication led to a consequent rise in cytosolic Ca^{2+}, that may activate cellular death pathways (54,55).

Therapy in ALS

Thus far, there is no therapy that has been proven to significantly benefit ALS patients. In Europe there is only the drug riluzole, that has been approved by the European Medicines Agency (EMA) for use in ALS patients, and its overall therapeutic benefit is questioned till this day. This is why symptomatic treatment (e.g. managing pain, sialorrhea and muscle spasticity) is given a lot of importance in the management of ALS (3,11,16).

Nonetheless, there are encouraging studies underway regarding the availability of much more effective and gene-targeted therapy that are RNA-based.

The neuroprotective agent: Riluzole

Riluzole is a synthetic benzothiazole (2-amino 6-(trifluoromethoxy)benzothiazole) that acts as a neuroprotective agent in the therapy of ALS (56).

Out of the randomized clinical trials (RCT) performed, it is shown that riluzole 100mg improves survival in ALS patients by about three months (57). Riluzole has short-term benefit as was shown in a retrospective study, whereby the mortality rate was reduced by 23% at six months from diagnosis and by 15% at twelve months from diagnosis, but this beneficial effect was lost within 18 months. This study also showed that bulbar-onset ALS patients benefitted more from riluzole than spinal-onset ALS. In addition, patients diagnosed with suspected or possible ALS (according to the El Escorial criteria) showed a 16% reduction in mortality rate at twelve months from diagnosis (58). Riluzole is well-tolerated and adverse drug reactions are not frequent. Elevated liver function tests, nausea and asthenia (lack of energy) are the commonest side-effects encountered (59).

RNA-targeted therapy in ALS

RNA-targeting therapeutics are evolving to be a major drug category and this enthusiasm is associated with the fact that this therapy is highly specific, when compared to the traditional drug categories of small molecules and proteins. Additionally, and importantly, these drugs are being studied for their use as a promising, efficient, and safe cure for ALS.

RNA-targeted therapy involves the use of synthetic oligonucleotides (a short chain of nucleic acids) and is based on the canonical Watson-Crick base pairing, exploiting, and targeting normal cellular components, such as endogenous nucleases. However, it is not this simple – structural and chemical modifications are required to enhance their effectiveness in the clinical setting.

When tackling the structure, one can either opt for antisense oligonucleotides (ASOs), which are single-stranded; or small interfering RNA (siRNA), which are double-stranded.

The emerging ASO in the therapy of ALS

Currently, there are undergoing clinical trials for an ASO therapy that targets the *SOD1* gene, which is one of the commonest genes to be affected in European ALS patients.

As previously mentioned, a damaging variant in *SOD1* gene results in misfolded aggregates of SOD1 protein, suggesting gain-of-function toxicity. Thus, lowering SOD1 protein levels is beneficial. This is exploited in the novel *SOD1*-targeting ASO: BIIB067, now known as Tofersen, which is an intrathecally administered ASO (60). The drug induces the degradation of *SOD1* mRNA by RNase H activity, thereby reducing the synthesis of SOD1 protein (61). Additionally, when tofersen was tested on mice expressing the $hSOD1^{G93A}$, a partial recovery in motor neuron function was exhibited (62).

A 28-week phase 3 randomised trial was conducted on tofersen, wherein the efficacy and safety were analysed in adults with *SOD1* ALS. It was discovered, that tofersen reduced concentrations of SOD1 in CSF, a marker of target engagement; and of plasma neurofilament light chains (NfL), a marker of neuronal degeneration. However, at 28 weeks, there was no significant difference in the change from baseline in the ALS Functional Rating Scale–Revised (ALSFRS-R) score between tofersen and the placebo group. These results suggest that early initiation of the drug may be more beneficial in delaying the onset of clinically manifest ALS. Thus, the ATLAS study is currently underway to evaluate the impact of tofersen when initiated in clinically presymptomatic adults with a confirmed *SOD1* mutation (63).

Also, tofersen was associated with mild to moderate adverse events, mostly associated with the disease progression of ALS and side effects of lumbar puncture. Nevertheless, 7% of the participants who received tofersen had serious neurological adverse events, including myelitis, meningitis and lumbar radiculopathy (64).

One must keep in mind that the *SOD1* gene is not damaged in the Maltese ALS cohort (7). This renders the drug ineffective for Maltese patients and this underscores the importance of the ongoing research concerning the unique genetic architecture in Maltese ALS patients. This research paves the way for the manufacture of efficient RNA-based therapy that is suited for the Maltese patients.

Conclusion

ALS is a devastating disease because of many aspects – it deprives the patients of their independence, it has poor prognosis, it can present in many forms and thus, is difficult to diagnose and it puts a severe strain on the healthcare system.

Clearly, there has been a dramatic advancement in the understanding of the genetic factors that are involved in ALS in the recent years. This will certainly continue to evolve, decoding more ALS genes as the vigorous research continues. Nevertheless, more research is required on the

environmental factors involved in the disease pathogenesis, since, as of now, such information is scarce. To do this, studies on ALS patients and on healthy controls are required. Several of such studies are already taking place, including Project MinE, which is an international ground-breaking study on ALS and healthy patient cohorts.

Thanks to the strong will and dedication of scientists, the manufacture of highly promising and efficacious cure is underway and should be available in the near future.

Declarations

Conflict of interest: N.A.

Ethical statement: N.A.

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