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Idiopathic Hypersomnia and Depression, the Challenge for Clinicians and Researchers

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Abstract: The review deals with idiopathic hypersomnia, focusing mostly on the research findings about the presence, onset and severity of excessive daytime sleepiness and depressive symptoms in patients with idiopathic hypersomnia.

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Introduction

Idiopathic hypersomnia (IH) was identified and named in the 20th century by renowned Czechoslovak neurologist, neurophysiologist and sleep researcher Bedřich Roth.

In 1956 the first description of IH appeared in Roth's article *Sleep drunkenness and sleep paralysis* in Czechoslovak Neurology. In 1976 Roth published the comprehensive description of the disorder. In the same publication the term idiopathic hypersomnia was used first and gained a general acceptance. We have come a long way since Roth's first work. However, there is still much work to be done. There is an absence of a sufficiently strong IH biomarker, poor epidemiological characterization, little knowledge of pathophysiology and no registered treatment.

The aim of this paper is to present the main findings concerning IH and to summarize the current and past information on depressive symptoms in IH. Because there is a discrepancy in the literature about the relationship between IH and depressive symptoms we reopen the existing, important question of whether the hypersomnolence in IH comes first or whether depressive symptoms (or mood disorder) are the first to appear. We speculate about the connection of pathophysiological aspects of both IH and mood disorders. This paper also discusses the problematic distinguishing among IH, hypersomnia associated with a psychiatric disorder and mood disorder.

Idiopathic hypersomnia – Current classification, diagnosis, differential diagnosis, and symptoms

Two main diagnostic manuals are commonly used to assist in diagnosing sleep disorders: the third edition of the International Classification of Sleep Disorders (ICSD-3) (American Academy of Sleep Medicine, 2014) and the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (American Psychiatric Association, 2013).

The ICSD-3 uses the term *idiopathic hypersomnia* (American Academy of Sleep Medicine, 2014) while according to the DSM-5 the disorder is called *hypersomnia* (American Psychiatric Association, 2013).

The ICSD-3 classifies IH as a central disorder of hypersomnolence (CDH). For the sake of completeness, it should be pointed out that the term *hypersomnolence* is used to name the symptom of excessive sleepiness, whereas *hypersomnia* refers to specific disorders, such as IH (American Academy of Sleep Medicine, 2014).

Hypersomnolence disorders may be divided into those that are *primary*, meaning they are not caused by another condition and disrupted night sleep namely narcolepsy type 1 (NT1) with cataplexy, narcolepsy type 2 (NT2) without cataplexy, IH and Kleine-Levin syndrome and those that are *secondary*, in which sleepiness is believed to be caused by the other disorders, medication, or short habitual sleep time.

Patients with IH suffer from periods of an irrepressible need for sleep or daytime lapses into sleep occurring for at least three months despite undisturbed nocturnal sleep of good quality. These periods occur according to the ICSD-3 daily (American Academy of Sleep Medicine, 2014), and according to DSM-5 at least 3 times a week (American Psychiatric Association, 2013). IH does not involve cataplexy. Sleep-onset REM period (SOREM) may occur on Multiple Sleep Latency Test (MSLT) and preceding night polysomnography (PSG) together less than twice. Objective evidence of hypersomnolence in IH must be demonstrated by MSLT showing the mean sleep latency (MSL) of ≤ 8 minutes, and/or by a total sleep time of ≥ 660 minutes/24 hours recorded by PSG or by wrist actigraphy and a sleep diary. In addition to that, sleep efficiency (SE) on PSG is usually above 90% in IH patients while self-reported quality of sleep may be poor. Patients typically do not easily wake up and often use special procedures or need someone who wakes them up (American Academy of Sleep Medicine, 2014).

Compared to narcolepsy, sleepiness in IH does not have an imperative character (Vernet et al., 2010). Hypersomnolence may fluctuate in severity and occurs typically in monotonous situations that require a low activation (while attending lectures, reading, watching TV), but may appear in situations demanding more attention (meeting at work or social event) as well. Naps are generally long, often longer than 60 minutes and described as unrefreshing by 46% to 78% of patients (American Academy of Sleep Medicine, 2014). The self-administered questionnaire Epworth Sleepiness Scale (ESS) is commonly used to measure the subjective excessive daytime sleepiness (EDS) (Johns, 1991).

Roth distinguished *monosymptomatic* and *polysymptomatic form* of IH (Roth, 1976). The *monosymptomatic form* manifests itself by EDS, while *polysymptomatic form* is characterized by EDS, prolonged night sleep usually of 12–18 hours duration and signs of sleep inertia historically known as *sleep drunkenness (SD)* upon awakening. Sleep drunkenness consists of prolonged and difficult period between awakening until the full wakefulness associated with repeated returns to sleep, disorientation, irritability and poor coordination (American Academy of Sleep Medicine, 2014).

Similarly to Roth (1976), the previous edition of the International Classification of Sleep Disorders (ICSD-2) distinguished two IH phenotypes as independent nosological units: IH with long sleep time (LST) (i.e. > 10 hours for the main sleep period) and IH without long sleep time (American Academy of Sleep Medicine, 2005).

The ICSD-3 merged two IH forms into one unit because of the insufficient diversity of these forms (Vernet and Arnulf, 2009; American Academy of Sleep Medicine, 2014). However, it has been suggested that IH with LST may be a juvenile form of the same disorder, later evolving towards less sleep in night.

In favour of this hypothesis is that total sleep time during night-time and during 24 hours slightly decreased with increasing age at diagnosis time (Vernet and Arnulf,

2009). A longitudinal follow-up of IH patients would be helpful to support this hypothesis.

Monosymptomatic form of IH is not very different from NT2. The only distinction between them is the presence of two or more SOREM periods on the MSLT or preceding PSG in NT2. Moreover, the SOREM occurrence is not stable in IH and NT2 (Trotti et al., 2013; Šonka et al., 2014; Lopez et al., 2017b; Ruoff et al., 2018). Thus IH (especially its monosymptomatic form) and NT2 may be the same condition (Lammers et al., 2020).

The differential diagnosis of IH consists primarily in the exclusion of chronic sleep deprivation, then other CDH, especially NT1 and NT2. EDS due to other sleep disorders, particularly obstructive sleep apnea (OSA) and periodic limb movement disorder (PLMD) must be also ruled out.

Mental illnesses, medication use, and substance abuse or head injury must be also considered and excluded (American Academy of Sleep Medicine, 2014). EDS must not be better explained by internal, autoimmune (Braga et al., 2016) and neurological disorders such as neurodegenerative diseases (Arnulf, 2005; Moreno-Lopez et al., 2011) or inflammatory diseases, particularly neuroinfections (Pardasani et al., 2008; Krbkova et al., 2015).

All the criteria for IH that must be met are shown in Table 1.

Epidemiology of IH and disease course

IH is a rare disorder, and its robust epidemiological studies are lacking. There is not valid data, but IH appears to be one-tenth to one-half as common as narcolepsy (Billiard, 1996; Bassetti and Aldrich, 1997; Anderson et al., 2007).

The age of symptom onset varies, but usually in adolescence or early adulthood (Anderson et al., 2007; American Academy of Sleep Medicine, 2014) although diagnosis is commonly delayed (Bassetti and Aldrich, 1997; Anderson et al., 2007).

For example, in the study of 77 IH patients, their mean age was 17 years at symptom onset and their mean age was 30 years at diagnosis (Anderson et al., 2007). Clinical experience suggests a higher incidence in women than in men (Roth, 1976; Bassetti and Aldrich, 1997; Anderson et al., 2007). The course of IH is not well studied, but once established, the disorder is generally stable in severity and long lasting (American Academy of Sleep Medicine, 2014), although a spontaneous remission has been reported in some IH patients (Bassetti and Aldrich, 1997; Anderson et al., 2007; Kim et al., 2016). However, factors that predict such remission remain unknown.

Etiology of IH

The term *idiopathic* is used to describe a disorder with no clear cause. One hypothesis assumes altered $GABA_A$ receptor inhibitory signalling in the etiology of IH (Rye et al., 2012). Other hypotheses consider the autoimmune mechanism (Barateau et al., 2017a; Lippert et al., 2019), the impact of infectious mononucleosis

Table 1 – Diagnostic criteria of idiopathic hypersomnia according to ICSD-3 (American Academy of Sleep Medicine, 2014)

Criteria A–F must be met:
A) The patient has daily periods of irrepressible need to sleep or daytime lapses into sleep occurring for at least three months.
B) Cataplexy is absent.
C) An MSLT performed according to standard techniques shows fewer than two sleep onset REM periods or no sleep onset REM periods if the REM latency on the preceding polysomnogram was less than or equal to 15 minutes.
D) The presence of at least one of the following: 1. The MSLT shows a mean sleep latency of ≤ 8 minutes. 2. Total 24-hour sleep time is ≥ 660 minutes (typically 12–14 hours) on 24-hour polysomnographic monitoring (performed after correction of chronic sleep deprivation), or by wrist actigraphy in association with a sleep log (averaged over at least seven days with unrestricted sleep).
E) Insufficient sleep syndrome is ruled out (if deemed necessary, by lack of improvement of sleepiness after an adequate trial of increased nocturnal time in bed, preferably confirmed by at least a week of wrist actigraphy).
F) The hypersomnolence and/or MSLT findings are not better explained by another sleep disorder, other medical or psychiatric disorder, or use of drugs or medications.

ICSD-3 – third edition of the International Classification of Sleep Disorders; MSLT – Multiple Sleep Latency Test

(Sforza et al., 2018) or abnormality of circadian rhythm in the pathophysiology of IH (Vernet and Arnulf, 2009; Lippert et al., 2014; Materna et al., 2018).

Depressive symptoms in IH

Up to now no questionnaire has been validated to assess the presence and the severity of depressive symptoms in patients with CDH (Lopez et al., 2017a). Screening questionnaires for depressive symptoms, such as the Beck Depression Inventory (BDI) is largely administrated in patients with CDH (Beck et al., 1961).

The Hamilton Depression Rating scale (HDRS), the Montgomery Asberg Rating scale (MADRS) or the Hospital anxiety and depression scale (HADS) are other commonly used instruments to quantify the severity of depressive symptoms (Hamilton, 1960; Montgomery and Asberg, 1979; Zigmond and Snaith, 1983).

We agree with Lopez and colleagues (2017a) that a structured (standardized) or semi-structured interview remain essential in the assessment of depressive symptoms in IH and hypersomnia associated with a psychiatric disorders.

Several studies have pointed to the presence of depressive symptoms in IH patients (Roth et al., 1971; Anderson et al., 2007; Vernet et al., 2010; Kim et al., 2016; Lee et al., 2017; Neikrug et al., 2017; Dauvilliers et al., 2019; Pascoe et al., 2019; Pomares et al., 2019). There is no consensus in the literature whether the

frequency of depressive symptoms is higher in IH patients or in narcoleptics. For example, in a study of Kim and colleagues (2016), the frequency of depressive symptoms was significantly higher in IH than in both NT1 and NT2.

In 1971 Roth and colleagues reported symptoms of recurrent depression in IH patients. Patients suffered from symptoms called by the authors as neurotic such as difficulties in concentration, emotional lability, and anxiety (Roth et al., 1971). Such findings are congruent with following investigation of Neikrug and colleagues (2017) because the most typical symptoms in IH patients were difficulty with concentration, irritability, loss of interest and anxiety. Recently, Trotti and colleagues (2020) reported in a web based questionnaire study symptoms such as *brain fog* defined as *being unable to think clearly or concentrate at any time throughout the day* and poor memory as a daily problem in most IH respondents.

However, there is no evidence on whether these additional symptoms are specifically related to IH either as a primary symptom more or less prominent or the consequence.

The studies with IH participants frequently lack healthy controls, as IH is usually compared with narcolepsy and the sample size of most of these studies is relatively small (Bassetti and Aldrich, 1997; Vernet and Arnulf, 2009). The lack of knowledge in this area may be caused by excluding IH patients from the study in case they have any coexistent psychiatric symptoms. Moreover, there is so little information about pediatric IH patients in the connection with depressive symptoms and thus IH in childhood will not be included in this review. Furthermore, according to our information, up to now no study has longitudinally examined depressive symptoms and their severity in IH patients.

IH, hypersomnia associated with a mood disorder and mood disorder – A diagnostic challenge

According to DSM-5, the hypersomnia is described in the association with mood disorders such as bipolar disorder (in depressive stage) or seasonal affective disorder. Patients with hypersomnia may suffer from depressive symptoms and may fulfil the diagnostic criteria for a mood disorder. The DSM-5 allows hypersomnia to be diagnosed independently from the presence of a current or past mood disorder because depressive symptoms may be connected with psychosocial consequences of EDS (American Psychiatric Association, 2013).

Contrary to the ICSD-3, the diagnosis of IH requires ruling out mood disorders. Patients with a psychiatric condition, most typically depression, should be diagnosed with hypersomnia associated with a psychiatric disorder. Nevertheless, the presence of depressive symptoms (not fulfilling the diagnostic criteria of the depression – disease) does not exclude the diagnosis of IH according to ICSD-3 (American Academy of Sleep Medicine, 2014).

Currently the differential diagnosis to distinguish IH from a mood disorder with EDS subjective symptoms relies heavily on the MSLT results. It is widely accepted,

that patients with a mood disorder do not have a short mean sleep latency of ≤ 8 minutes (Billiard and Dauvilliers, 2001; American Academy of Sleep Medicine, 2014). The complaint of EDS and prolonged sleep may be similar in patients with IH, but in mood disorders the degree of EDS is not stable over time. The SE on PSG in IH patients is usually above 90% while in mood disorders the night sleep tends to be of poor quality (Billiard and Dauvilliers, 2001).

The differential diagnosis relies on MSLT results to distinguish IH from hypersomnia associated with psychiatric disorder as well. The study comparing the night-time sleep duration between the group of patients with a hypersomnia associated with a psychiatric disorder and the group of IH revealed that 14% of psychiatric hypersomniacs slept over 9 hours at night and 36.1% of psychiatric hypersomniacs had a reduced MSL (Billiard et al., 1994). Such findings are congruent with a recent meta-analysis of Plante (2017).

Lammers and colleagues (2020) disagree with a current ICSD-3 classification and suggest entities such as hypersomnia associated with a psychiatric disorder, hypersomnia due to medical disorder, hypersomnia due to substance abuse should be discontinued because in most cases it is not presently known if the relationship is truly causal or simply co-morbid (Barateau et al., 2017b; Plante, 2017). Instead, medical disorders and psychiatric disorders including substance abuse should be considered and listed as possible co-morbidities. This would be in line with decisions made by ICSD-3 to allow a diagnosis of insomnia independently from the presence of a psychiatric disorder, medical condition, drug or substance intake (Lammers et al., 2020).

It would be also in line with DSM-5 allowing a diagnosis of hypersomnia independently from the presence of a current or past psychiatric disorder (American Psychiatric Association, 2013).

EDS in mood disorders

The evidence suggests the frequency of EDS is high in mental health disorders and particularly in mood disorders (Detre et al., 1972; Akiskal and Benazzi, 2005; Kaplan and Harvey, 2009; Lopez et al., 2017a; Plante et al., 2017).

EDS in major depressive disorder (MDD) significantly varies across age, ranging from 8.9% in childhood (<13 years) to a high rate of 75.8% in young adulthood. EDS also varies across gender, but appears to be more prevalent in females than in males (Kaplan and Harvey, 2009). The large frequency range likely reflects nonuniform definitions of EDS and assessing the EDS by subjective questionnaires only, or simple questions on sleep duration.

According to Dauvilliers and colleagues (2013), EDS in mood disorders is a subjective sleep complaint rather than an objective finding. Dysthymic patients did not show abnormalities on the MSLT, neither on the PSG compared to a group of IH patients. However, dysthymic patients showed an excess of sleep stage NREM 1 and a decrease of stages NREM 3 and 4, which could be related to their complaint of

EDS (Dolenc et al., 1996). Importantly, since the update of the classification of sleep stages (the American Academy of Sleep Medicine – AASM) in 2007, NREM 3 and NREM 4 are combined as stage N3 (Iber et al., 2007).

It seems that these patients tended to remain in bed without an objective evidence for increased sleep time. This behaviour is known as clinophilia (American Academy of Sleep Medicine, 2014).

The chronology of onset of depression and EDS

The chronology of onset of mood disorders and EDS could help to clarify the relationship between these two conditions. In the case of suspicion of IH and the presence of depressive symptoms, it is relevant to study the date of onset of each symptom, the evolution of EDS depending on mood and the effect of the treatment of depressive symptoms on EDS and vice versa.

There is still a lack of knowledge about IH in a clinical practice, it is possible that due to general knowledge about mood disorders, some patients with IH are misdiagnosed and treated as depression and diagnosis of IH is thus delayed.

EDS in mood disorders is commonly considered to be a consequence of the disorder, in the line with monoamine activity disturbances (especially catecholamines, dopamine or serotonin) (Lopez et al., 2017a; Ogawa et al., 2018). The meta-analysis of Ogawa and colleagues (2018) revealed that homovanillic acid levels (often used as an indicator of dopaminergic activity) were decreased in the cerebro-spinal fluid of patients with a mood disorder (Lambert et al., 1993; Ogawa et al., 2018).

The study of Roth (1956) shown that depressive symptoms occurred at the very same time as EDS. Also, Bassetti and Aldrich (1997) reported that EDS began in the association with prominent psychiatric complaints with parallel fluctuations of mood and EDS in IH patients with a positive family history for a mood disorder.

Some studies agree with the idea that EDS precedes or is concomitant rather than subsequent to mood disorders because non-depressed subjects with a family history of mood disorder commonly have disturbances of REM sleep (Jaussent et al., 2011; Kaplan et al., 2011). Specifically, the shorter REM latency and increased REM sleep density were associated with the development of mood disorder during follow-up (Rao et al., 2009). The view that shortened REM sleep latency could be a specific marker of mood disorders has been frequently debated (Kupfer and Foster, 1972; Coble et al., 1981; Kupfer et al., 1986).

However, this sleep pattern cannot be used to diagnose of mood disorder because of the great variability in depressed patients (Kupfer and Foster, 1972; Lauer et al., 1991).

The possible aetiologic relationship between IH and depression

We assume there are four possible relationships between IH and depressive symptoms.

Firstly, depressive symptoms in IH patients might be a consequence of a difficultly adapting to the chronic disorder (Bassetti and Aldrich, 1997; Vernet and Arnulf, 2009; Lopez et al., 2017a). Patients with IH often report poor academic and work performances (Bassetti and Aldrich, 1997; Anderson et al., 2007; Avis et al., 2015; Neikrug et al., 2017) and increased incidence of car or work accidents (Bassetti and Aldrich, 1997; Ozaki et al., 2008; Pizza et al., 2015). EDS also affects social and family life and thus decreases quality of life (Ozaki et al., 2008, 2012; Avis et al., 2015; Neikrug et al., 2017; Miglis et al., 2020). This might lead to a depressive mood and to intensification of depressive symptoms or result in a mood disorder later (Lopez et al., 2017a). However, even after the treatment of EDS with stimulants, depression scores may or may not improve, suggesting that the relationship between IH and depressive symptoms is more complex than one of mere cause and effect.

Secondly, depressive symptoms might be a possible symptom of the pathological process that causes IH. In the study of Vernet and colleagues (2010) half of patients reported a stressful event before the onset of IH, for example the death of a loved one, divorce, a serious illness, the end of the military service or the end of a high level of sport practice. In addition to that, 23% of them had insomnia and 38% of them had a major change in sleep habits before the onset of IH (Vernet et al., 2010). In our opinion the structure of personality, the overall mood and depressive symptoms may contribute to development of IH.

Thirdly, IH and mood disorders might be two disorders that often occur together.

Lastly, EDS might be a symptom of depression and the disease that we call IH might be a variant phenotype of depression. Nevertheless, we do consider that this option is not probable because there are IH subjects without any mood disturbances and there is not an important pathophysiological link suggesting this speculation.

Conclusion

IH is frequently connected with depressive symptoms (or mood disorders) yet the etiopathogenetic relationship is unclear. It is indeed possible that the relationship between IH and depressive symptoms (or mood disorders) may be more complex than just the result of the handicapping disorder. While research concerning this area is ongoing, we ought to make time to listen to IH patients and improve symptoms at least symptomatically.

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Valproate-associated Movement Disorder: A Literature Review

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Abstract: Valproate (VPA) was first synthesized in 1882, but it was only in the early 1960s that its anticonvulsant properties were discovered. The aim of this literature review is to evaluate the clinical epidemiological profile, pathological mechanisms, and management of VPA-associated movement disorder (MD). Relevant reports in six databases were identified and assessed by two reviewers without language restriction. A total of 138 reports containing 362 cases of subjects who developed a MD secondary to VPA were reported. The MD identified were parkinsonism (PKN) (252), myoclonus (MCL) (54), dystonia (DTN) (17), dyskinesia (DKN) (16), stutters (4), tics (3), akathisia (AKT) (1). In the not clearly defined group, 15 extrapyramidal symptoms, 3 AKT, 2 DTN, 1 rigidity, 1 unstable gait were assessed. The mean and median age was 55.8 (SD: 16.58) and 61 years (range: 4–87 years). The most common VPA-indication was epilepsy, and 51.36% were males. The mean and median time from the VPA start to the MD onset was 32.75 (SD: 30.05) and 21.15 months (range: 1 day – 20 years). The mean and median time from the VPA withdrawal until the MD recovery was 2.89 (SD: 2.79) and 3 months (1 day – 12 months). The most common management was drug withdrawal. A complete recovery was obtained in 80.61%. VPA-associated MD was extensively reported in the literature. PKN was the most well-described. Future studies need to clearly report the clinical history of the patient, considering the full investigation of other adverse events during their entire life.

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Introduction

Valproate (VPA), and its pharmacological forms such as valproic acid, sodium valproate, and valproate semisodium are anticonvulsants (Figure 1). In 1882, Beverly Burton synthesized VPA for the first time; this compound was used for almost eighty years as an inert solvent in laboratories (Lempérière, 2001). Pierre Eymard, in the early 1960s, during animal studies to develop a new antiepileptic drug, noted that the substances dissolved in VPA had apparently better anticonvulsant properties (Henry, 2003). After this observation, many clinical studies showed the efficacy and safety of VPA for the management of focal seizures (Brugger et al., 2016). In 1967, it was approved as an antiepileptic drug in France (Henry, 2003). Only in 1983, the Food and Drug Administration approved this medication for the treatment of epilepsy (Lempérière, 2001). The first study assessing the efficacy of VPA in bipolar disorder was done by Lambert et al. at the end of the 1960s in France, soon after the approval for epilepsy, which showed good results, but for many years these data were believed to be incidental, due to the small number of subjects studied (Henry, 2003). About ten years later, German clinical trials followed by North American studies supported the hypothesis of Lambert et al. In 1995, VPA was approved as monotherapy during manic episodes by the FDA (Lempérière, 2001).

The mechanism of action of VPA is not completely understood (Figure 2) (Lempérière, 2001; Löscher, 2002; Bowden, 2003; Henry, 2003; Brugger et al., 2016). Its main interactions are related to the voltage-gated sodium channels blockage and increased brain levels of gamma-aminobutyric acid (GABA) (Löscher, 2002). The increased concentration of this neurotransmitter is believed to occur due to indirect inhibition of the GABA's reuptake and degradative enzymes. Also, it is worth mentioning that this GABAergic mechanism probably explains the anticonvulsant and antimanic properties of this drug (Bowden, 2003). Other pathways that VPA is related include the Kv7.2, AKAP5, and histone deacetylase (Löscher, 2002). It is hypothesized that the inhibition of the histone deacetylase may have neuroprotective effects due to the increased uncoiling of DNA promoting more transcriptional activity of chromatin structures (Bowden, 2003).

The adverse effects of this medication that affect more than ten percent of users are nausea, vomiting, headache, coagulation disorders (Rissardo et al.,

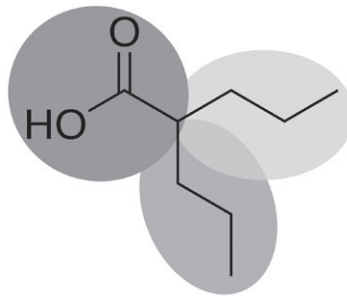

Figure 1 – Skeletal formula of the anticonvulsant drug valproic acid, also known as 2-propylvaleric acid.

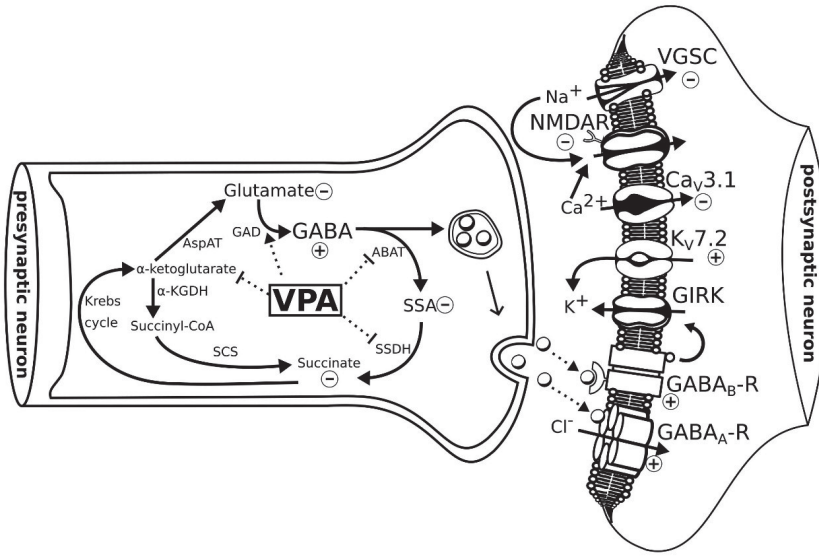

Figure 2 – Representation of proposed mechanisms of action for valproate (VPA). α-KGDH – alpha-ketoglutarate dehydrogenase; ABAT – 4-aminobutyrate aminotransferase; AspAT – aspartate aminotransferase; CaV3.1 – voltage-gated calcium channel; GABA – gamma-aminobutyric acid; GABAA/B-R – GABAA/B receptor; GAD – glutamate decarboxylase; GIRK – G protein-gated inwardly rectifying potassium channel; KV7.2 – voltage-gated potassium channel; NMDAR – N-methyl-D-aspartate receptor; SCS – succinyl CoA synthetase; SSA – succinic semialdehyde; SSDH – succinate-semialdehyde dehydrogenase; VGSC – voltage-gated sodium channel.

2019), alopecia, asthenia, somnolence, amblyopia, diarrhea, dizziness, dyspepsia, nystagmus, and tinnitus (Bowden, 2003). In the label of VPA, there is a black box warning about hepatotoxicity in susceptible individuals (those with mitochondrial diseases), teratogenicity, and pancreatitis (Löscher, 2002). Other common side effects secondary to VPA are movement disorders (MD) such as tremor and ataxia, which can significantly impact the quality of life of an important percentage of the VPA users. Moreover, these abnormal movements are challenging to diagnose and manage in the clinical practice, because the majority of affected individuals have a pre-existing psychiatric or neurologic comorbidity.

In the literature, there are few reviews about VPA and MD that were not focused solely on tremors. To be more specific, we found two reviews about VPA-induced parkinsonism (PKN). Mahmoud and Tampi published a study about this topic in 2011; they objectively selected elderly patients, and a total of thirteen case reports were analysed. In 2016, Brugger et al. searched on four databases for papers in English about VPA and PKN, a total of 116 patients were evaluated; their purpose was to discuss the possible hypotheses for these adverse effects. In this context, the aim of the present literature review is to evaluate the clinic-epidemiological profile, pathological mechanisms, and management of VPA-associated MD.

Methods

Search strategy

We searched six databases and also abstracts of the "International Congress of the Parkinson's Disease and Movement Disorders (1990–2019)" in an attempt to locate any and all existing reports on movement disorders (MD) secondary to VPA published between 1975 and 2019 in electronic form. Excerpta Medica (Embase), Google Scholar, Latin American and Caribbean Health Sciences Literature (Lilacs), Medline, Scientific Electronic Library Online (Scielo), and ScienceDirect were searched. Search terms were "parkinsonism, dyskinesia, dystonia, stuttering, myoclonus, restless legs syndrome, akathisia, tremor, chorea, tics, restlessness, ataxia, ballism, hyperkinetic, hypokinetic, bradykinesia, movement disorder". These terms were combined with "valproate, valproic acid" (Table 1).

Inclusion and exclusion criteria

Case reports, case series, original articles, letters to the editor, bulletins, and poster presentations published from 1975 to 2019 were included in this review with no language restriction. The authors independently screened the titles and abstracts

Table 1 – FreeText and MeSH search terms in the US National Library of Medicine

Category	Search terms	Results
Parkinsonism	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((((((((“parkinson disease”[MeSH Terms] OR (“parkinson”[All Fields] AND “disease”[All Fields])) OR “parkinson disease”[All Fields]) OR “parkinson’s”[All Fields]) OR “parkinsons”[All Fields]) OR “parkinson”[All Fields]) OR “parkinsonian disorders”[MeSH Terms]) OR (“parkinsonian”[All Fields] AND “disorders”[All Fields])) OR “parkinsonian disorders”[All Fields]) OR “parkinsonism”[All Fields]) OR “parkinsonisms”[All Fields]) OR “parkinsons’s”[All Fields])	180
Tics	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (“TIC”[Journal] OR “TIC”[All Fields])	15
Dyskinesia	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((“dyskinesiae”[All Fields] OR “dyskinesias”[MeSH Terms]) OR “dyskinesias”[All Fields]) OR “dyskinesia”[All Fields]) OR “dyskinesis”[All Fields])	576

Category	Search terms	Results
Dystonia	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (((((“dystonia”[MeSH Terms] OR “dystonia”[All Fields]) OR “dystonias”[All Fields]) OR “dystonic disorders”[MeSH Terms]) OR (“dystonic”[All Fields] AND “disorders”[All Fields])) OR “dystonic disorders”[All Fields])	54
Stuttering	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((((((((“stammerers”[All Fields] OR “stammers”[All Fields]) OR “stutterer”[All Fields]) OR “stutterer’s”[All Fields]) OR “stutterers”[All Fields]) OR “stuttering”[MeSH Terms]) OR “stuttering”[All Fields]) OR “stammer”[All Fields]) OR “stammering”[All Fields]) OR “stutter”[All Fields]) OR “stuttered”[All Fields]) OR “stutters”[All Fields]) OR “stutterings”[All Fields])	10
Myoclonus	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (((“myoclonias”[All Fields] OR “myoclonus”[MeSH Terms]) OR “myoclonus”[All Fields]) OR “myoclonia”[All Fields])	345
Restless legs syndrome	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((“restless legs syndrome”[MeSH Terms] OR ((“restless”[All Fields] AND “legs”[All Fields]) AND “syndrome”[All Fields])) OR “restless legs syndrome”[All Fields])	17
Akathisia	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((“akathisias”[All Fields] OR “psychomotor agitation”[MeSH Terms]) OR (“psychomotor”[All Fields] AND “agitation”[All Fields])) OR “psychomotor agitation”[All Fields]) OR “akathisia”[All Fields])	122
Tremor	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (((((“trembling”[All Fields] OR “tremor”[MeSH Terms]) OR “tremor”[All Fields]) OR “tremors”[All Fields]) OR “tremoring”[All Fields]) OR “tremorous”[All Fields])	222
Chorea	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((“chorea”[MeSH Terms] OR “chorea”[All Fields]) OR “choreas”[All Fields])	124

Category	Search terms	Results
Restlessness	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((“psychomotor agitation”[MeSH Terms] OR (“psychomotor”[All Fields] AND “agitation”[All Fields])) OR “psychomotor agitation”[All Fields]) OR “restlessness”[All Fields]) OR “restless”[All Fields])	124
Ataxia	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((“ataxia”[MeSH Terms] OR “ataxia”[All Fields]) OR “ataxias”[All Fields])	205
Ballism	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((“dyskinesias”[MeSH Terms] OR “dyskinesias”[All Fields]) OR “ballism”[All Fields])	542
Hyperkinetic	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (“hyperkinetic”[All Fields] OR “hyperkinetics”[All Fields])	20
Hypokinetic	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((“hypokinesia”[MeSH Terms] OR “hypokinesia”[All Fields]) OR “hypokinetic”[All Fields])	9
Bradykinesia	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND (((“hypokinesia”[MeSH Terms] OR “hypokinesia”[All Fields]) OR “bradykinesia”[All Fields]) OR “bradykinesias”[All Fields])	12
Movement disorder	((((((“valproate”[All Fields] OR “valproate’s”[All Fields]) OR “valproates”[All Fields]) OR “valproic acid”[MeSH Terms]) OR (“valproic”[All Fields] AND “acid”[All Fields])) OR “valproic acid”[All Fields]) OR “valproate”[All Fields]) AND ((((“movement disorders”[MeSH Terms] OR (“movement”[All Fields] AND “disorders”[All Fields])) OR “movement disorders”[All Fields]) OR (“movement”[All Fields] AND “disorder”[All Fields])) OR “movement disorder”[All Fields])	466
Total		**3043**

of all papers found in the initial search. Disagreements between the authors were resolved through discussion.

Cases where the cause of MD was already known and either motor symptoms did not worsen or were not related to VPA were excluded. Also, cases that were not accessible by electronic methods, even after a formal request to the authors (by e-mail) were excluded. Reports that had more than one factor contributing to the MD were evaluated by the probability of occurrence of the event based on the Naranjo algorithm.

Data extraction

For VPA a total of 6,443 papers were found; 5,279 were irrelevant and 1,026 were unrelated to the complication, duplicate, inaccessible electronically, or provided insufficient data (Figure 3). Data abstraction was performed. When provided, we extracted author, department, year of publication, country of origin, number of patients affected, VPA indication including off-label uses, time from first VPA-dose until MD onset, time from VPA withdrawal or management to symptoms improvement, patient's status at the last follow-up, and important findings of clinical history and management. The majority of the reports did not provide specific

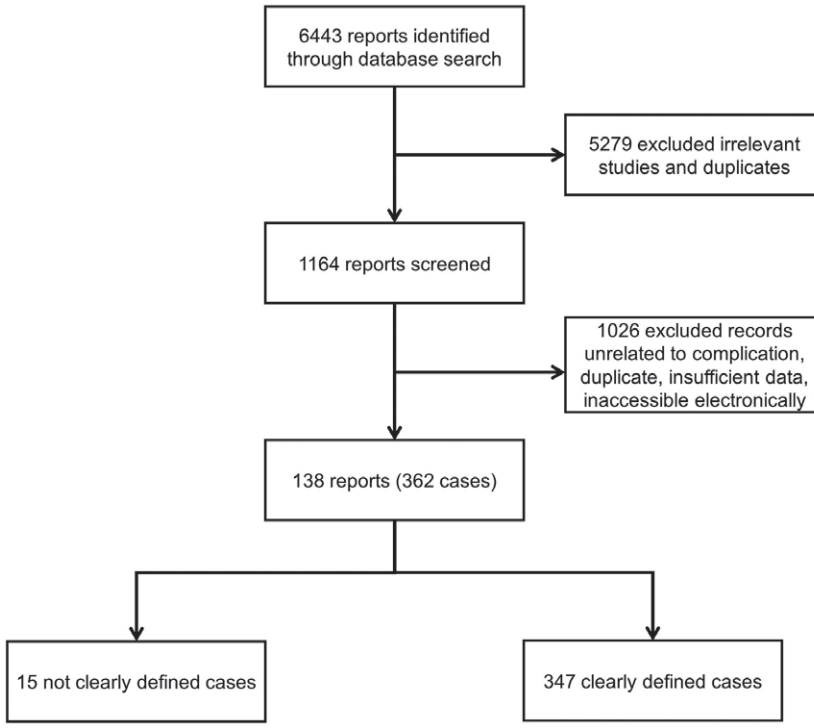

Figure 3 – Flow chart of the screening process for valproate (VPA).

information about the times of MD onset and recovery. Data were extracted by two independent authors, double-checked to ensure matching, and organized by whether or not the MD was a side effect of VPA use.

Statistical analysis
Categorical variables were represented as proportions; continuous variables were represented as mean, standard deviation (SD), median, and range.

Definitions
The clinical characteristics and definitions of the MDs such as parkinsonism, tics, dyskinesia, dystonia, myoclonus, restless legs syndrome, akathisia, tremor, chorea, ataxia, and ballism were obtained from the reference Jankovic and Tolosa (2007). The clinical diagnosis for the psychiatric conditions was obtained from the Diagnostic and Statistical Manual of Mental Disorders (DSM-5®) (American Psychiatric Association, 2013). The Naranjo algorithm was used for determining the likelihood of whether an adverse drug reaction was actually due to the drug rather than the result of other factors (Naranjo et al., 1981). In the cases where the non-English literature was beyond the authors' proficiency (English, Portuguese, Spanish, Italian, French, and German) and the English abstract did not provide enough data, such as Japanese, Korean, Chinese, Russian, and Dutch, Google Translate service was used (De Vries et al., 2018).

Results

For the years 1975 to 2019, a total of 138 reports containing 362 cases, from thirty-three countries, of individuals who developed a movement disorder (MD) secondary to valproate (VPA) were reported (Table 2). Figure 4 shows the number of reports

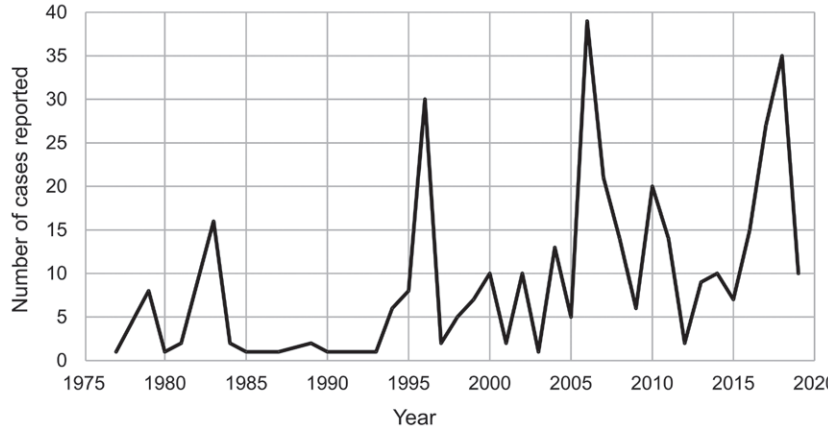

Figure 4 – Graphic showing the number of clinical reports of valproate (VPA)-associated movement disorders (MD) from 1975 to 2019.

Table 2 – Clinical reports of VPA-associated MD

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
MYOCLONUS									
Lance and Anthony	Australia 1977	1	adult/F	EPI	1200	months	NA	NA	
Nutt et al.	USA 1979	1	57/M	PD	1000–2000	33 days	NA	NA	CH: asterixis CM: drug withdrawal
Bodensteiner et al.	USA 1981	2	NA	EPI	NA	NA	NA	NA	
Campostrini et al.	Italy 1983	2	adult/ 1 M + 1 F	EPI	NA	NA	NA	NA	CH: hyperammonemia
Zaccara et al.	Italy 1984	2	19.5/ 1 M + 1 F	EPI	800 (mean)	2 weeks (mean)	4 days	CR	CH: asterixis, one cortical, other subcortical; apparently threshold-effect. CM: in one patient the MCL appeared after a dose increase. The dose was reduced but the symptoms persisted. VPA withdrawal with symptoms recovered
Gastaut and Mege	France 1985	1	adult/ NA	EPI	NA	NA	NA	NA	CH: hyperammonemia without hepatic insufficiency
Aguglia et al.	Italy 1995	6	39.83/ 3 F + 3 M	EPI	1200	1 week	2–6 days	CR	CH: cortical asterixis. CM: VPA withdrawal
Vogt and Mothersill	Switzerland 2000	3	43/3 F	EPI	NA	NA	NA	CR	CH: subcortical, EEG-based. Possible interaction with oxcarbazepine and lamotrigine. CM: drug withdrawal

Shirasaka and Mitsuyoshi	Japan 1999	1	10/F	EPI	NA	1 month	weeks	CR	CH: cortical asterixis; dose-dependent effect: dose increase caused an increase of MCL frequency: VPA withdrawal
Rottach et al.	Germany 2000	1	28/NA	BD	600	2 weeks	4 days	CR	CH: cortical asterixis, EEG-based. CM: VPA withdrawal
Kao et al.	Taiwan 2001	1	51/F	EPI	1500	2 weeks	< 1 week	CR	CH: subcortical asterixis; hyperammonemia; possible interaction with CBZ. CM: VPA withdrawal
Reif et al.	Germany 2004	1	42/M	DPS	2000	7 day	2 days	CR	CH: cortical MCL, EEG-based. CM: VPA withdrawal
Brefel-Courbon et al.	France 2006	20	NA	NA	NA	NA	NA	NA	
Dealberto and Sarazin	Canada 2008	1	41/F	mood	1000	1 year	2 days	CR	CH: cortical asterixis, EEG-based; hyperammonemia. CM: VPA withdrawal
Fan et al.	Taiwan 2008	1	72/F	mood	900	3 months	1 week	CR	CH: cortical asterixis, EEG-based; possible interaction with lamotrigine; hyperammonemia. CM: VPA withdrawal
Yoon et al.	Korea 2008	1	56.7/M	EPI	NA	NA	NA	NA	
Gardner et al.	USA 2009	1	66/F	mood	750	NA	NA	CR	CH: multifocal MCL. CM: VPA withdrawal
Mangewala et al.	USA 2013	1	17/M	EPI	1500	5 months	NA	no (only improve-ment	CH: asterixis; hyperammonemia. CM: VPA-dose decrease with partial improvement of the symptoms
Nayak et al.	India 2012	1	35/F	EPI	1000	2 weeks	< 6 weeks	CR	CH: subcortical asterixis; possible interaction with antipsychotics. CM: VPA withdrawal

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Surendran et al.	India 2016	1	57/M	BD	1500–2000	7 years	3 weeks	CR	CH: cortical asterixis, EEG-based; hyperammonemia; possible interaction with risperidone. CM: VPA withdrawal
DYSTONIA									
Dick and Saunders	USA 1980	1	adult/M	DTN	600–1800	days–weeks	NA	NA	CH: worsening of cervical + axial DTN. CM: VPA withdrawal
Kiuru and Iivanainen	Finland 1987	1	23/F	EPI	1200–1500	months	1 week	CR	CH: axial DTN that occurred after a dose increase. Previous history of hepatopathy due to CBZ + VPA + lynestrenol interaction. CM: VPA-dose decreased with the resolution of the symptoms
Dunayevich and Strakowski	USA 1999	1	40/F	schizo-phrenia	1250	7 months	NA	no	CH: choreoathetotic DKN + (blepharospasm + oromandibular + cervical) DTN; possible interaction with olanzapine; previous history of blepharospasm with loxapine. CM: olanzapine-dose decrease and clozapine started with partial recovery
Oh et al.	Korea 2004	1	40/M	EPI	1200	days	1 week	CR	CH: spasmodic dysphonia. CM: VPA-dose reduction with the recovery of the symptoms
Teive et al.	Brazil 2004a	1	8/M	EPI	NA	NA	NA	NA	CH: VPA was present when the status DTN occurred

Werner et al.	Switzerland 2006	1	elderly/ NA	NA	NA	NA	NA	NA	CH: possible differential with DTN; dropped head syndrome was more severe in patients using VPA
Yohanan et al.	USA 2006	1	65/M	schizo-phrenia	1500	several months	weeks	CR	CH: axial DTN; possible interaction with CBZ + risperidone. CM: VPA withdrawal
Habermeyer et al.	Switzerland 2007	1	60/F	mood	900	4 days	NA	CR	CH: axial DTN (anterocollis). CM: VPA and quetiapine-dose decrease and biperiden started with symptoms recovery
Lee et al.	Korea 2007	1	64/F	EPI	600	days	NA	NA	CH: patient with probable Creutzfeldt-Jakob disease that worsened DTN and MCL when VPA was started
Zadikoff et al.	Canada 2007	2	47.5/ 1 F + 1 M	EPI	NA	NA	NA	NA	CH: one task specific DTN, other hemi-DTN
Czarnecki et al.	USA 2008	1	59/M	BD	NA	days	NA	no	CH: axial DTN (anterocollis) + PKN + AKT; possible interaction with risperidone + quetiapine. CM: all drugs withdrawal without improvement; he received the diagnosis of frontotemporal dementia
Duggal	USA 2008	1	20/M	schizo-phrenia	1000	3 days	1 day	CR	CH: axial DTN (laterocollis); possible interaction with ziprasidone. CM: benztropine was started with an improvement of the symptoms
Bayram et al.	Turkey 2013	1	4/F	EPI	NR	6 months	NA	CR	CH: cervical DTN; possible interaction with butamirate citrate

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Faridhosseini et al.	Iran 2015	1	33/F	schizo-phrenia	1000	months	1 month	CR	CH: axial DTN; possible interaction with clozapine. CM: clozapine-dose reduced and biperiden started
Bermudez et al.	Brazil 2017	1	62/F	BD	1000	3 days	NA	CR	CH: trismus, possible oromandibular DTN. CM: biperiden and clonazepam were prescribed without response. VPA withdrawal with symptoms resolution
DYSKINESIA									
Friis et al.	Denmark 1983	3	32.33/1 F + 2 M	several	1400 (mean)	NA	NA	NA	CH: all the subjects had DKN associated with some degree of AKT
Lancman et al.	USA 1994	3	21/2 F + 1 M	EPI	1466 (mean)	4 years	NA	CR	CH: 2 individuals were in use of phenytoin, possible interaction. CM: cessation of VPA or the substitution to VPA sprinkles improved the symptoms
Gara and Roberts	Canada 2000	2	5.37/1 F + 1 M	EPI	1125	years	NA	CR	CH: orofacial DKN, but oromandibular DTN cannot be excluded; possible interaction with methylphenidate. CM: Pt1 VPA withdrawal; Pt2 methylphenidate replaced by clonidine
Gunal et al.	Turkey 2002	1	38/M	EPI	1500	2 months	2 months	CR	CH: choreiform DKN. CM: VPA withdrawal

Hall and Ringel	USA 2004	1	42/F	nonketotic hyper-glycinemia	NA	NA	NA	NA	CH: chorea in the presence of VPA after protein loading; the combination of chronic VPA administration and a meal high in protein could have contributed to the DKN
Morrison et al.	USA 2006	1	11/F	nonketotic hyper-glycinemia		NA	5 days	NA	CH: choreiform DKN. CM: VPA withdrawal
Srinivasan and Lok	Singapore 2010	1	53/M	EPI	1900	months	2 days	CR	CH: hemichoreiform DKN; the MD occurred after one week of VPA-dose in a dose higher than the prescribed. CM: VPA withdrawal; after VPA rechallenge without the occurrence of new symptoms
van de Velde et al.	Belgium 2011	1	80/F	migraine	1200	4 years	days-weeks, < 6 months	CR	CH: choreiform DKN, weeks after the VPA-dose increase. CM: VPA withdrawal and haloperidol started
Yilmaz et al.	Turkey 2013	1	7/M	EPI	500	NA	NA	CR	CH: orofacial DKN after 2 days in the use of methylphenidate + VPA
Giordano et al.	Italy 2014	1	53/F	EPI	900	days	1 week	CR	CH: choreiform DKN + axial DTN. CM: VPA withdrawal
Bruno et al.	UK 2016	1	36/M	EPI	1000	single IV	1 week	CR	CH: choreiform DKN + multifocal MCL. CM: VPA withdrawal
					STUTTER				
Bowdle et al.	USA 1979	2	22/NA	none	1000–1500	days–weeks	NA	NA	CH: the adverse effects started appearing at 1,000 mg. The speaking was characterized as stammering

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Mukherjee et al.	India 2015	1	56/M	BD	1500	1 week	< 1 week	CR	CH: his articulation, intensity, timings of utterance, rhythm were affected. CM: VPA withdrawal; VPA rechallenge caused symptoms reappearance
AKATHISIA									
Clos	Scotland 2001	1	38/F	BD	1200	1 month	NA	no	CH: possible interaction with lithium. CM: VPA withdrawal with partial improvement of the symptoms
TIC									
Alonso-Navarro et al.	Spain 2007	1	14/M	EPI	1500	1 day	NA	CR	CH: motor + phonic tics. CM: ziprasidone started with the improvement of the symptoms
Zadikoff et al.	Canada 2007	1	47/M	EPI	NA	NA	NA	NA	CH: excessive eye blinking
Thome-Souza et al.	Brazil 2012	1	18/F	EPI	1000	1.2 years	NA	CR	CH: motor tics; possible interaction with lamotrigine. CM: lamotrigine and VPA-dose decrease with symptoms improvement
PARKINSONISM									
Lautin et al.	USA 1979	1	52/M	schizo-phrenia	1000	4 days	2 days	CR	CH: he had a history of metoclopramide and haloperidol developing PKN. CM: benztropine and trihexyphenidyl started with symptoms permanence. VPA was withdrawal

Nutt et al.	USA 1979	4	56.6/2 F + 2 M	PD	2000	28 days	NA	NA	CH: the four patients developed worsening of PKN features. Two developed MCL. CM: drug withdrawal; after the study, the doses of these subjects affected needed to be significantly increased
Friis et al.	Denmark 1983	11	37/1 F + 10 M	several	1700	NA	NA	NA	CH: all the subjects had some level of AKT associated to the PKN. 5 subjects had PKN + DKN
van der Zwan	Australia 1989	2	adult/NA	EPI	NA	NA	NA	CR	
Power et al.	Canada 1990	1	adult/NA	EPI	NA	NA	NA	NA	
Alvarez-Gomez et al.	Spain 1993	1	12/F	EPI	600	7 days	1 month	CR	CM: VPA replaced by CBZ with symptoms resolution
Froomes and Stewart	Australia 1994	1	67/F	EPI	1400	months	3 days	CR	CH: possible interaction with CBZ. CM: CBZ withdrawal with symptoms resolution
Sasso et al.	Italy 1994	2	23/2 M	EPI	1250	16 months	5 weeks	CR	CM: VPA maintenance and symptoms improved over time
del Real Francia et al.	Spain 1995	2	70.5/2 F	NA	1500	years	weeks	CR	CM: VPA withdrawal
Armon et al.	USA 1996	27	51.5/NA	EPI	1400	44.8 months	< 3 months	CR (96%)	CM: VPA withdrawal
Gwinn and Caviness	USA 1997	1	69/M	BD	500	11 months	NA	no	CH: PKN + orofacial DKN; possible interaction with risperidone. CM: drug withdrawal with the permanence of DKN

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Onofrj et al.	Italy 1998	2	65.5/1 F + 1 M	EPI	1150	6.5 years	< 3 months	CR	CH: in one of the patients a dose increase was reported 6 months before the MD onset; patients assessed by Unified Parkinson's Disease Rating Scale; possible interaction with CBZ
Park-Matsumoto and Tazawa	Japan 1998	3	73/2 F + 1 M	EPI	800	21 months	4.3 months	CR	
Conforti et al.	Italy 1999	1	69/F	BD	1200	< 3 months	days–weeks	CR	CH: possible interaction with nortriptyline + venlafaxine. The PKN started with the added of nortriptyline. CM: nortriptyline-dose reduce improved the symptoms
Lapierre et al.	Canada 1999	1	62/F	BD	1400	days–weeks	4 days	CR	CH: possible PKN marked stiffness and motor slowness. CM: VPA-dose reduced with a full recovery
Nouzeilles et al.	Argentina 1999	3	60.33/NA	EPI	1259	NA	NA	NA	
Kim et al.	Korea 2000	1	69/F	EPI	1200	2 months	< 1 month	CR	
Masmoudi et al.	France 2000	5	64/2 F + 3 M	EPI	1400	6 months–10 years	weeks–months	CR	CH: dementia characterized by an insidious onset was associated in three cases and bradypsychia in one case. CM: VPA withdrawal
Shill and Fife	USA 2000	1	67/F	EPI	NA	2 years	3 months	CR	CH: multiple system atrophy-like syndrome. CM: VPA withdrawal

Barroso	France 2002	1	72/NA	NA	NA	NA	NA	NA	
Foley et al.	USA 2002	6	70/6 M	3 EPI + 3 BD	1166.7	21.3 months	NA	CR	CH: gait abnormality was the most common presenting symptom
Iijima	Japan 2002	1	77/M	behavior control	300	1 week	NA	CR	CM: VPA withdrawal
Raja and Azzoni	Italy 2002	1	38/NA	NA	NA	NA	NA	CR	CH: possible PKN and interaction with quetiapine
Reif et al.	Germany 2003	1	62/F	mood	1200	10 days	7 days	CR	CH: possible interaction with lithium; hyperammonemia. CM: VPA and lithium withdrawal; amantadine started
Easterford et al.	UK 2004	5	60.2/4 M + 1 F	EPI	1220	> 1 year	NA	CR (2/5)	CH: normal β-CIT-SPECT. CM: VPA withdrawal
Ferrari et al.	Italy 2004	3	52.66/3 M	EPI	1250	4.6 year	NA	CR	CH: one of the patients had PKN after IV VPA; dose-unrelated side effect
Lee	Korea 2004	1	61/M	BD	1500	days–weeks	< 7 days	CR	CM: PKN partially relieved by the coadministration of anticholinergic agents and disappeared after discontinuation of VPA
Dergalust et al.	USA 2005	1	adult/NA	NA	NA	NA	NA	NA	
Macphee	Scotland 2005	1	73/M	mood	1500	3 months	3 months	CR	CH: FP-CIT SPECT scan was normal. CM: VPA withdrawal
Ricard et al.	France 2005	1	58/M	BD	1000	7 months	1 month	CR	CH: PKN + cognitive impairment + hyperammonemia. CM: VPA withdrawal
Thygesen and Wolf	Denmark 2005	2	64/1 F + 1 M	EPI	1400	10 months	weeks	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Gaubert et al.	France 2006	1	82/M	EPI	2000	17 months	3 weeks	CR	CM: VPA withdrawal

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Ristić et al.	Serbia 2006	5	59.9/4 F + 1 M	EPI	1100	12.9 months	NA	no (only improvement)	CH: early identification of PKN and discontinuation of the drug led to complete recovery in affected patients
Borroni et al.	Italy 2007	1	61/F	EPI	1200	10 years	NA	no (only improvement)	CH: multiple system atrophy-like syndrome. CM: VPA withdrawal
Hommet et al.	France 2007	1	81/M	EPI	500	2 months	3 months	CR	CH: possible PKN. CM: VPA-dose decrease without improvement; VPA withdrawal with full recovery
Jamora et al.	USA 2007	6	42.16/4 F + 2 M	EPI	750	76.16 months	NA	no (only improvement)	
Macphee and Stewart	Scotland 2007	1	67/F	EPI	600	10 years	NA	no (only improvement)	CH: FP-CIT SPECT scan was normal. CM: VPA withdrawal
Zadikoff et al.	Canada 2007	6	44.5/4 F + 2 M	EPI	1425	7.33 years	NA	NA	
Aguilar and Ondo	USA 2008	4	63.2/NA	NA	NA	71.4 months	NA	NA	
Maximov and Maximov	Bulgaria 2008	1	75/M	EPI	2000	NA	NA	No	CM: levodopa was started with improvement of the symptoms
Salazar et al.	Argentina 2008	1	67/M	mood	1000	days	2 months	CR	CH: PKN + axial DTN (laterocollis); a history of Huntington's disease. CM: VPA withdrawal

Sechi et al.	Italy 2008	1	39/F	palatal MCL	1000	3 months	1 month	CR	CH: a history of Alexander's disease. CM: VPA withdrawal
Toribio-Díaz et al.	Spain 2008	1	78/F	EPI	1500	2 months	2 months	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Abreu et al.	Brazil 2009	1	47/F	BD	2500	7 months	NA	no (only improve-ment)	CH: PKN + cognitive impairment. CM: VPA withdrawal
Lidbom et al.	Sweden 2009	1	72/F	mood	1400	11 years	NA	NA	CH: PKN + cognitive impairment. CM: VPA withdrawal
Louter and Tromp	Netherlands 2009	1	57/F	NA	NA	NA	NA	NA	
Schreur et al.	Netherlands 2009	2	70/2 M	EPI	850	2.5 years	NA	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Andrade et al.	Cuba 2010	1	52/F	EPI	1140	2 months	2 months	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Khwaja et al.	India 2010	1	26/F	EPI	1000	2 years	3 months	CR	CH: progressive supranuclear palsy-like syndrome. CM: VPA withdrawal
Lyell et al.	UK 2010	2	76/NA	NA	NA	NA	NA	NA	
Munhoz et al.	Brazil 2010	13	67.6/NA	NA	NA	NA	NA	NA	
Penot and Pradeau	France 2010	1	75/F	EPI	1000	1 week	2 months	CR	CH: possible PKN + axial DTN and interaction with aspirin. CM: VPA-diose decrease with the improvement of the symptoms
Sleegers et al.	Netherlands 2010	1	70/F	EPI	1000	months	5 weeks	CR	CH: PKN + cognitive impairment; a history of systemic lupus erythematous. CM: VPA withdrawal
Bondon-Guitton et al.	France 2011	10	NA	NA	NA	NA	NA	NA	

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Evans et al.	USA 2011	1	65/F	EPI	1000	13 years	NA	no (only improvement)	CH: PKN + cognitive impairment; normal pressure hydrocephalus-like presentation. CM: VPA withdrawal
Sarna and Pringsheim	Canada 2011	1	63/F	BD	NA	1–2 years	NA	no (only improvement)	CH: progressive supranuclear palsy-like syndrome. CM: VPA withdrawal
Gosala Raja Kukkuta et al.	India 2013	1	56/M	EPI	800	6 months	4 days	CR	CH: progressive supranuclear palsy-like syndrome; hyperammonemia. CM: VPA withdrawal
Silver and Factor	USA 2013	5	63/3 F + 2 M	2 DPS + 1 MCL + 1 BD + 1 EPI	1000	1.81 months	9 months	CR (3/5)	CH: PKN + cognitive impairment (3/5). CM: VPA withdrawal
Athauda et al.	UK 2015	1	65/M	EPI	3000	8 years	NA	no (only improvement)	CH: due to continuous PKN signs after management a DaTSCAN was performed which showed abnormal uptake and he received the diagnosis of idiopathic PD. CM: VPA withdrawal
Jopowicz and Kurkowska-Jastrzebska	Poland 2014	1	76/M	EPI	1000	3 months	NA	no (only improvement)	CM: VPA-dose reduce with the improvement of the symptoms
Prakash et al.	Denmark 2015	1	76/F	EPI	1200	years	NA	no (only improvement)	CH: PKN + cognitive impairment. CM: VPA withdrawal
Desai and Desai	India 2015	3	NA	NA	NA	NA	NA	NA	

Hassamal et al.	USA 2016	1	71/F	BD	1250	8 years	12 months	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Irons et al.	UK 2015	1	87/M	NA	NA	NA	NA	no (only improvement)	CH: multiple system atrophy-like syndrome; DaTSCAN positive. CM: VPA withdrawal
Bhattacharjee et al.	UK 2016	1	54/M	BD	NA	NA	NA	no (only improvement)	CH: PKN that persisted after management; DaTSCAN positive. CM: VPA withdrawal
Botturi et al.	Italy 2016	1	59/M	EPI	900	3 months	3 months	CR	CH: axial DTN + PKN; hyperammonemia. CM: VPA withdrawal
Simões et al.	Portugal 2016	8	72/6 M + 2 F	6 EPI + 2 psychitric condition	1379	4 months	8 months	CR	CM: VPA withdrawal
Tada et al.	Japan 2017	1	75/F	BD	1000	6 months	NA	no (only improvement)	CH: PKN + cognitive impairment. CM: VPA withdrawal
Bhattacharjee et al.	UK 2017	24	68/NA	NA	NA	NA	NA	NA	assessment of the DAT-SPECT scans conducted for the diagnosis of drug-induced versus idiopathic PKN
Caruana Galizia et al.	UK 2017	1	60/F	EPI	1700	20 years	2 weeks	CR	CH: PKN + cognitive impairment; symptoms occurred after the withdrawal of phenytoin, so possible interaction cannot be ruled out. CM: VPA withdrawal

Reference	Country/ year	No. of cases	Age (mean)/ sex	VPA				Follow-up	Important clinical history (CH) and clinical management (CM)
				indication	dose (mg)	MD onset	MD recovery		
Aykut et al.	Turkey 2018	1	72/M	BD	2500	10 years	6 days	CR	CH: PKN + axial DTN. CM: VPA withdrawal
Kim et al.	Korea 2018	1	69/F	EPI	1200	3 years	1 week	CR	CH: 18F-FP-CITPET was normal. CM: VPA withdrawal
Patel et al.	India 2018	1	58/M	EPI	500	7 years	NR	CR	CM: VPA withdrawal
Yomtoob et al.	USA 2018	17	64.54/10 F + 7 M	NA	NA	4.3 years	NA	NA	
Baizabal-Carvallo and Alonso-Juarez	Mexico 2021	2	35.9/NA	EPI	1365	NA	NA	NA	
Bennet and Rosen	USA 2019	2	71/2 F	1 BD + 1 DPS	NA	6 months	NA	CR	CH: PKN + cognitive impairment. CM: VPA withdrawal
Dal and Whyte	Australia 2019	2	77.5/2 M	EPI	1200	2.5 years	NA	no (only improvement)	CH: PKN, after withdrawal the patients did not have recover; levodopa was started and the PKN alleviated. CM: VPA withdrawal
Davoudi-Monfared et al.	Iran 2019	1	54/M	mood	1500	NA	weeks	CR	CH: possible PKN + cognitive impairment. CM: VPA withdrawal
Randhawa and Mehanna	USA 2019	1	NA	BD	NA	3 months	NA	no (only improvement)	CH: possible interaction with risperidone; normal DAT-SPECT scan even after the permanence of PKN symptoms
Kohlhase et al.	Germany 2019	2	61/2 M	EPI	1525	years	NA	CR	CH: PKN; 1 hyperammonemia; normal DAT-SPECT scan. CM: VPA withdrawal

CASES NOT CLEARLY DEFINED				
Leclair-Visonneau et al.	France 2016	NA	PKN	Assessment of the efficacy of VPA in progressive supranuclear palsy. The progressive supranuclear palsy rating scale at 12 months was significantly higher in the VPA than in the placebo group but was similar between the two groups at 24 months. According to the authors' this strongly suggests poor tolerability due to side effects than permanent neurological damage.
Wang et al.	Germany 2016	NA	EPS, DTN, AKT, rigidity	Assessment of the efficacy of VPA in schizophrenia. The studies compared VPA + antipsychotic to antipsychotic; none of the adverse effects were significant. They encountered AKT (3/186, RR 1.06 [0.36, 3.06]); ataxia (2/115, RR 2.42 [0.37, 15.92]); DTN (2/130, RR 1.00 [0.30, 3.37]); rigidity (1/33, RR 2.83 [0.12, 64.89]); unstable gait (1/19, RR 0.27 [0.02, 3.39]). Interesting, the VPA was associated with a significant decrease of DKN of –3.31 (–4.91, –1.71).
Makhlouf et al.	Tunisia 2018	15	EPS	Assessment of the incidence of VPA side effects in a Tunisian population. 15 subjects complained of slowness of execution of movements, and EPS was observed in every one of these subjects.

AKT – akathisia; BD – bipolar disorder; CBZ – carbamazepine; CH – clinical history; CM – clinical management; CR – complete recovery; DKN – dyskinesia; DPS – depression; DTN – dystonia; EEG – electroencephalogram; EPI – epilepsy; EPS – extrapyramidal symptoms; F – female; M – male; MCL – myoclonus; MD – movement disorder; NA – not applicable/not available; PKN – parkinsonism; PD – Parkinson's disease; RR – risk ratio; SPECT – single-photon emission computed tomography; VPA – valproic acid/valproate

Table 3 – Resume of VPA-associated MD

MD		PKN	MCL	DTN	DKN	Stutter	Tic	AKT	Others	General data
Cases (%)		252 (69.61)	54 (14.91)	17 (4.69)	16 (4.41)	4 (1.10)	3 (0.82)	1 (0.27)	15 (4.14)	362
Continent (%)	Africa	0	0	0	0	0	0	0	15 (100)	15
	Australia	0	1 (1.85)	0	0	0	0	0	0	1
	Asia	17 (6.74)	6 (11.11)	4 (23.52)	3 (18.75)	1 (25.00)	0	0	0	31
	Europe	122 (48.41)	36 (66.66)	3 (17.64)	6 (37.50)	1 (25.00)	1 (33.33)	1 (100)	0	170
	N. America	90 (35.71)	11 (20.37)	8 (47.05)	7 (43.75)	2 (50.00)	1 (33.33)	0	0	119
	S. America	23 (9.12)	0	2 (11.76)	0	0	1 (33.33)	0	0	26
Sex (%)	Female	74 (29.36)	15 (27.77)	8 (47.05)	8 (50.00)	0	1 (33.33)	1 (100)		107
	Male	83 (32.93)	10 (18.51)	8 (47.05)	8 (50.00)	2 (50.00)	2 (66.66)	0		113
	Unknown	95 (37.69)	29 (53.70)	1 (5.88)	0	2 (50.00)	0	0		142
Age (y)	Range	12–87	10–72	4–65	5.37–80	22–56	14–47	38		4–87
Mean	60.87	40.85	40.26	30.67	36.25	26.33	38		55.88 (SD: 16.58) Mn: 32.75 mo (SD: 30.05) Mn: 2.89 mo (SD: 2.79)	(Md: 61)
VPA-dose (Mn mg)		1315	1223	1113	1274	1150	1250	1200		1296 (SD 365; Rg 300–3000; Md 1379)
*VPA-indication		EPI (123/172)	EPI (27/34)	EPI (5/14)	EPI (10/18)	BD (1/4)	EPI (3/3)	BD (1/1)		EPI (170/246)
MD onset	Range	4 d – 20 y	7 d – 7 y	3 d – 7 mo	1 d – 4 y	4 d – wks	1 d – 1.2 y	1 mo		1 d – 20 y
Mean (y)	3.38	0.49	0.23	2.21	0.02	0.60	1 mo			(Md: 21.15 mo)
MD recovery	Range	2 d – 12 mo	2 d – 6 wk	1 d – 1 mo	2 d – 2 mo	3 d – 1 wk	NA	NA		1 d – 12 mo
Mean (mo)	3.41	0.33	0.40	0.95	0.16	NA	NA			(Md: 3 mo)
Follow-up – % CR (number of reports)		77.02% (114/148)	95.23% (20/21)	81.81% (9/11)	100% (11/11)	100% (2/2)	100% (2/2)	0% (0/1)		80.61% (158/196)

AKT – akathisia; BD – bipolar disorder; CR – complete recovery; d – day; DKN – dyskinesia; DTN – dystonia; EPI – epilepsy; MCL – myoclonus; MD – movement disorder; Md – median; Mn – mean; mo – month; NA – not available/not applicable; PKN – parkinsonism; SD – standard deviation; Rg – range (minimum–maximum); VPA – valproic acid/valproate; y – year; wk – week; in the "Others" subgroup are cases not specified about the movement disorder such as extrapyramidal symptoms; *main VPA-indication (no/total)

of VPA-associated MD over time. There were 170 reports from Europe, 119 North American, 31 Asian, 26 South American, 15 African, and 1 Australian. The MDs identified were parkinsonism, with 252 cases, 54 cases of myoclonus, 17 of dystonia (DTN), 16 of dyskinesia, 4 of stutters, 3 of tics, and 1 of akathisia (AKT). In the "not clearly defined group", 15 cases of extrapyramidal symptoms, 3 of AKT, 2 of DTN, 1 of rigidity, and 1 of unstable gait were assessed.

The summary data about VPA-associated MD is provided in Table 3. Herein, we will describe the general data of all clearly defined cases.

The abnormal movements occurred in males in 51.36% of the cases. The mean and median age was 55.8 (SD: 16.58) and 61 years (age range: 4–87 years). The indication of VPA in descending order of frequency was epilepsy 69.10% (170/246), bipolar disorder (25), "mood related" (12), DKN (dyskinesia) in Parkinson's disease (5), schizophrenia (5), depression (4), myoclonus (MCL) (2), DTN (1), migraine (1), twitch eyelids (1), and others non-specified psychiatric conditions (20).

The mean and median time from starting VPA use to the MD onset was 32.75 (SD: 30.05) and 21.15 months (MD onset time range: 1 day – 20 years), respectively. About 75% of the individual had abnormal movement within 50 months of the VPA treatment. The mean and median time from the VPA withdrawal until the MD recovery was 2.89 (SD: 2.79) and 3 months (MD recovery time range: 1 day – 12 months), respectively. In the subgroup of subjects that had improvement of the symptoms, the complete recovery was achieved within 9 months of the drug withdrawal in almost all cases (99%). Figure 5 shows a comparison between the percentage of patients who developed a MD since the beginning of the treatment and the percentage of patients recovering after drug withdrawal when outliers were removed.

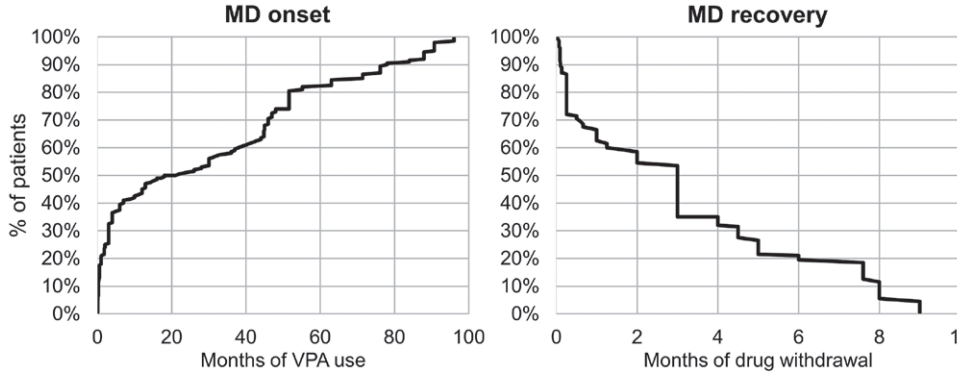

Figure 5 – Comparison between the percentage of patients developing movement disorders (MD) since the beginning of the drug treatment and the percentage of patients recovering after drug withdrawal.

The most common management was drug withdrawal. Other options were the VPA-dose reduction, replacement of the drug probably interacting with VPA, and the prescription of other drugs after the VPA discontinuation, such as levodopa, benztropine, benzodiazepines, biperiden, haloperidol, and diphenhydramine. In addition, the replacement of VPA in tablet form for the same dosage in sprinkles presentation was sufficient to improve the symptoms in one case. A complete recovery was observed in 80.61% of the patients (158/196).

Discussion

General

VPA-associated MD was widely reported in the literature. We believe that the availability, costs, and some historical factors of VPA probably had contributed to this. VPA is among the safest and most effective medicines needed in a health system, as attested by the World Health Organization's List of Essential Medicines, and it is marketed in the majority of countries. Also, VPA was the 126th most prescribed medication in the USA with almost six million prescriptions in 2017 (ClinCalc, 2020). Furthermore, the well-known description of flunarizine and cinnarizine developing PKN in 1984, promoted the awareness of the drug-induced MD resulting in an increasing number of reports about all abnormal movements secondary to medications including those associated with VPA (Teive et al., 2004b).

Based on the data available in Table 2, we can hypothetically illustrate a case. A middle-aged European male with poorly controlled epilepsy resorts to his neurologist. VPA 250 mg with a gradual increase until five-six tablets a day was prescribed. Within three years, the patient started complaining of stiffness, rigidity, and resting tremor; neurological examination revealed bradykinesia, and a diagnosis of PKN secondary to VPA was done. VPA was established and lamotrigine or carbamazepine was started. In the follow-up after three months, the patient had a full recovery and was able to walk without assistance and the tremor disappeared.

The majority of the incidences of abnormal movements associated with VPA are not well described in the literature. Table 3 is a summary of the percentages of some abnormal movements secondary to VPA (Anthony, 1977; Bowdle et al., 1979; Friis et al., 1983; Zaccara et al., 1984; van der Zwan, 1989; Armon et al., 1996; Nouzeilles et al., 1999; Easterford et al., 2004; Ristić et al., 2006; Jamora et al., 2007; Zadikoff et al., 2007; Lance and; Leclair-Visonneau et al., 2016; Makhlouf et al., 2018; Baizabal-Carvallo and Alonso-Juarez, 2021); the data was extracted from the clinical trials and population-based studies that provide sufficient data for Table 2. The incidences of VPA-associated abnormal movements extensively vary throughout the literature. For example, VPA-induced PKN was observed from 1.37 to 75% of the individuals.

Herein, we would like to discuss some of the MDs in subtopics to allow a better comprehension of the data.

Parkinsonism (PKN)

History

In 1979, Lautin et al. reported the first case of VPA-induced PKN. They described a middle-aged male who was prescribed VPA 1,000 mg for schizophrenia; four days later, the patient complained of PKN symptoms. Benztropine and trihexyphenidyl were started, but the symptoms did not alleviate. Only when VPA was withdrawn the patient had a full recovery. Also, the individual had a previous history of PKN secondary to metoclopramide and haloperidol. Therefore, we believe that this has contributed significantly to the literature because a similar presentation of VPA and antidopaminergic drugs in the same individual suggested a common neuronal pathway associated with extrapyramidal symptoms of these two drug classes.
It is worth mentioning that in the same year Nutt et al. (1979) published the cases of four individuals with Parkinson's disease with worsening gait and resting tremors that were using VPA.

Epidemiology

The incidence of PKN following VPA use found in the literature was 1.37, 1.60, 2.27, 5.04, 6.00, 10.16, 10.71, 73.33, and 75.00% (Table 4). The majority of the individuals reported were males, the mean age was 60.87 years, the mean VPA-dose was 1,315 mg. The time since starting VPA until MD onset, and the time until resolution after VPA discontinuation were 3.38 years and 3.41 months, respectively. When we compare the present study with the Brugger et al. (2016); the main differences encountered are that the present work has assessed a greater number of patients (252 vs. 116), of which the majority was male (52.86% vs. 41.4%); interestingly, the findings of Brugger et al. (2016) for mean age (63.5 years), VPA main indication (epilepsy) and median VPA dose (1,250 mg) were almost identical to those already described in this revision.

Presentation and clinical diagnosis

The presentation in the majority of the cases was a symmetric akinetic-rigid syndrome, with predominant postural/action over the resting tremor. Sometimes signs and symptoms of cognitive impairment were observed. The severity of the clinical presentation ranged from mild to severe with loss of physical independence. Some patients had pre-existing diseases other than the indication for VPA prescription such as Parkinson's disease, multiple system atrophy, progressive supranuclear palsy, Huntington's disease, systemic lupus erythematosus, and some brain damage. Interestingly, multiple system atrophy-like and progressive supranuclear palsy-like syndromes were reported as the presenting symptoms.
A clear distinction between VPA-induced MD and idiopathic Parkinson's disease based only on clinical criteria is challenging in clinical practice. Therefore, we proposed some clinical tools to help with the diagnosis of this syndrome (Table 5).

Table 4 – Incidence of some abnormal movements associated with VPA in the literature

MD	Reference	Year	NR	N	Incidence (%)	Studied disease
MCL	Lance and Anthony	1977	1	60	1.66	EPI
Ataxia	Lance and Anthony	1977	1	60	1.66	EPI
Tremor	Lance and Anthony	1977	2	60	3.33	EPI
Stutter	Bowdle et al.	1979	2	6	33.33	healthy
AKT	Friis et al.	1983	15	15	100.00	several
PKN	Friis et al.	1983	11	15	73.33	several
DKN	Friis et al.	1983	8	15	53.33	several
MCL	Zaccara et al.	1984	2	38	5.26	EPI
PKN	van der Zwan	1989	2	88	2.27	EPI
PKN	Armon et al.	1996	27	36	75.00	EPI
Tremor	Armon et al.	1996	16	36	44.44	EPI
Bradykinesia	Armon et al.	1996	22	35	62.85	EPI
PKN	Nouzeilles et al.	1999	3	28	10.71	EPI
Intentional tremor	Nouzeilles et al.	1999	15	28	53.57	EPI
Postural tremor	Nouzeilles et al.	1999	16	28	57.14	EPI
PKN	Easterford et al.	2004	3	50	6.00	EPI
Tremor	Easterford et al.	2004	11	50	22.00	EPI
PKN	Ristić et al.	2006	5	364	1.37	EPI
Tremor	Ristić et al.	2006	28	364	7.69	EPI
Ataxia	Ristić et al.	2006	7	364	1.92	EPI
PKN	Jamora et al.	2007	6	119	5.04	EPI
PKN	Zadikoff et al.	2007	6	59	10.16	EPI
Postural/ action tremor	Zadikoff et al.	2007	6	59	10.16	EPI
DTN	Zadikoff et al.	2007	2	59	3.38	EPI
Tic	Zadikoff et al.	2007	1	59	1.69	EPI
Worsening of the gait	Leclair-Visonneau et al.	2016	3	28	10.71	PSP
Movements slowness	Makhlouf et al.	2018	15	74	20.27	EPI
PKN	Baizabal-Carvallo and Alonso-Juarez	2021	2	125	1.60	EPI/migraine

AKT – akathisia; DKN – dyskinesia; DTN – dystonia; EPI – epilepsy; MCL – myoclonus; MD – movement disorder; N – number of individuals in the study using VPA; NR – number of reports with the movement disorder; PKN – parkinsonism; PSP – progressive supranuclear palsy; VPA – valproic acid/valproate

Table 5 – Clinical tools for the diagnosis of VPA-induced PKN

1) History of VPA use
2) PKN after therapy with VPA use (at least two of the following symptoms: bradykinesia, rigidity, and postural instability)
3) Usually, symmetrical PKN with prominent action/postural over resting tremor
4) More commonly affects elderly individuals
5) Recovery with VPA withdrawal
6) Other possible causes of PKN excluded

PKN – parkinsonism; VPA – valproic acid/valproate

Pathophysiological mechanism

In the literature, we found five possible pathophysiological mechanisms to explains the VPA-induced PKN (Figure 6) (Brugger et al., 2016). First, the VPA can increase the concentration of GABA (Löscher, 2002), which inhibits the globus pallidus connections with the thalamus, decreasing activity of the direct pathway. Second, another effect of VPA is the inhibition of the histone deacetylase that may increase the expression of some genes and decrease others such as those involved in the synaptic transmission (Löscher, 2002), which was already suggested in cell studies. Third, most affected individuals were elderly, so they may already have an imbalance of dopaminergic and cholinergic activity, and when VPA is used, a decrease in dopamine occurs, favoring the indirect pathway (Sawle et al., 1990). Fourth, VPA can, in a normal concentration of neurotransmitters (balanced state), affect mitochondrial enzymes causing cellular energy deficiency, what increases the

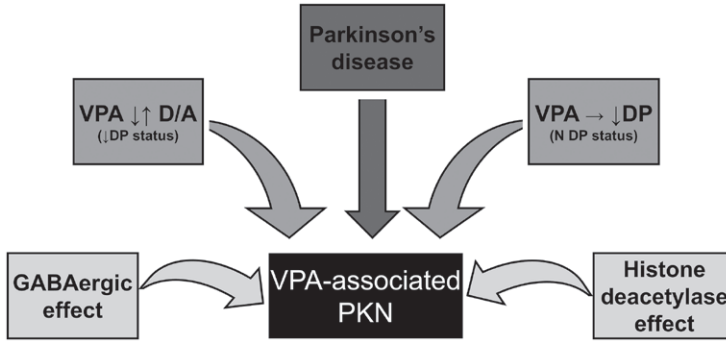

Figure 6 – Schematic diagram of the possible pathophysiological mechanisms to explains the valproate (VPA)-induced parkinsonism (PKN). The first pier represented by the GABAergic and histone deacetylase effects, which are directly related to the mechanism of action of valproate (VPA). The second pier is dependent on the dopamine (DP) status of the patient that could be decreased (↓) or normal (N), in which the presence of VPA can causes a disbalance (↓↑) of dopaminergic and cholinergic activity (D/A). The third pier is those individuals with coexisting Parkinson's disease.

likelihood of an oxidative stress and consequently a neurodegenerative process, especially in the dopaminergic system (Löscher, 2002); a supporting fact for this theory is that individuals with particular mitochondrial lesions are more susceptible to the development of side effects related to VPA (Henry, 2003). Fifth, the diagnosis of Parkinson's disease in a significant percentage of the patients cannot be ruled out; as a result, perhaps the use of VPA was only by chance present in these individuals, who may develop uncorrelated Parkinson's disease.

Management

The most commonly reported management was the VPA discontinuation, adopted in more than seventy-five percent of the individuals. In some cases, dopamine precursors were attempted to manage and a partial improvement of the PKN symptoms was achieved; in the follow-up, levodopa showed to be effective and reduced the recovery time, but apparently these cases were after diagnosed with Parkinson's disease. One individual received bromocriptine, but no details were provided regarding treatment response. The VPA-induced PKN had the second-worst prognosis, full recovery was obtained in 77.02% of the subjects; about 10% of subjects only had partial improvement of the symptoms, with permanence of at least one symptom even after the last follow-up.

Myoclonus (MCL)

MCL was the first VPA-associated MD identified and was the second most commonly reported in the literature. The incidence of MCL related to VPA use found in the literature was 1.66–5.26% (Table 4). MCL-individuals were approximately twenty years younger than those affected by PKN, also, the VPA dose was lower, and MD onset and recovery happened sooner than in general data. The majority of the subjects involved were female (3:2). The presentation was asterixis and multifocal MCL. The MCL source was cortical and subcortical. It is worth mentioning that an important percentage of the cases only describe the neurological examination, giving the diagnosis without providing the findings of the electrodiagnostic studies. The management was drug withdrawal or the reduction of VPA-dose.

A feature reported in an important percentage of the patients, and possibly related to the mechanism of VPA-induced MCL, is the high serum concentrations of ammonia, with no sign of liver failure described in the eight individuals assessed. Therefore, some authors believe that the explanation for MCL in the group of hyperammonemic individuals is the decrease of inhibitory neurotransmitters caused by ammonia, turning the individuals more susceptible to the development of MCL (Campostrini et al., 1983; Gastaut and Mege, 1985). On the other hand, the possibility that the ammonia levels found in these cases may be incidental cannot be excluded, as clinical trials with VPA already reported higher levels of this compound in individuals without any complaint (Löscher, 2002; Bowden, 2003). Also, this

hypothesis can support the idea that perhaps VPA action on the central nervous system may lead to the development of MCL. Moreover, we hypothesized that the mechanism behind VPA-induced MCL is probably related to VPA interaction with serotonin. In rat models, VPA caused both increase and decrease in serotonin concentration, depending on the site of action (Baf et al., 1994).

Dystonia (DTN)

In the DTN group, the data obtained for doses and times until onset and recovery from MD are comparable to general data on drug-induced DTN found in literature. Dick and Saunders (1980) probably described the first case of VPA-associated DTN. They reported the case of an individual with cervical and axial DTN that they attempted to treat with VPA, and the DTN-symptoms worsened.

The presentation in descending order of frequency was axial, cervical, oromandibular, blepharospasm, status dystonicus, and spasmodic dysphonia. We included the spasmodic dysphonia in the DTN group, but some authors believe that this disorder is a different entity, which goes beyond the aim of this review (Oh et al., 2004). In the same way, dropped head syndrome commonly reported with anticonvulsants may or may not be related to DTN (Werner et al., 2006). Possible interactions with clozapine, risperidone, quetiapine, and butamirate citrate were described.

One of the possible assumptions to explain the VPA-induced DTN is based on the GABAergic neurotransmission (Löscher, 2002). We believe that due to increased GABA levels by VPA the direct and indirect pathways that go to the thalamus might be interrupted. But the indirect pathway subactivity could probably predominate, and this disruption can increase the thalamocortical drive and eventually lead to DTN (Rissardo and Caprara, 2019). Another hypothesis related to dopaminergic activity in a mechanism similar to that proposed for the VPA-induced PKN can also be assumed (Brugger et al., 2016).

Dyskinesia (DKN)

Friis et al. (1983) reported in 53.33% of the individuals receiving VPA the development of DKN (Table 4). The presentation was orofacial, choreiform, hemichoreiform, and choreoathetotic. The association with another MD was observed with axial DTN and multifocal MCL. The DKN, stutter, and tics had the best prognosis with 100% recovery after the management.

The effects of VPA in the dopaminergic system probably explain the VPA-induced DKN. One fact that can support this hypothesis is the long time from starting VPA treatment until the MD onset, which was after 2.21 years. It is believed that due to the dopamine blockage, antipsychotics trigger inflammatory processes and the release of reactive oxygen species causing abnormal adaptations of the striatal organization, and ultimately leading to overactivation of the direct pathway (Lepping et al., 2011).

The most frequent management was VPA withdrawal. Another option was the VPA-dose reduction in those individuals that a possible interaction related to protein intake or other medications was assumed. Moreover, Lancman et al. (1994) reported in one subject the substitution from VPA tablet to sprinkles improving the symptoms. A possible relation with the higher VPA plasma concentration and the DKN occurrence can be proposed in this case, since the sprinkles have their peak within four hours and other VPA formulation in one hour, especially the syrup (Cloyd et al., 1992).

Stutter, tic, and akathisia (AKT)

In our analysis, we included stutter because of the possible differential diagnosis of DTN, MCL, and even DKN due to poor description of the neurological examination. It was observed only in young adult males with bipolar disorder. The MD's times of onset and recovery were the shortest, and the VPA-dose was the lowest reported in relation to the general data. These features can support the assumption of possible DTN diagnosis. Also, the prognosis was excellent, with 100% recovery. The most effective treatment was the drug withdrawal. Mukherjee et al. (2015) attempted the VPA reintroduction, which caused the reappearance of the symptoms. Thus, in the VPA-induced stuttering, we believe that the rechallenge of VPA should not be done.

Tics were observed in three individuals and corresponded to less than one percent of VPA-induced MD reports. In the data extraction of the Zadikoff et al. (2007) study, the percentage of individuals developing tics with VPA use was 1.69%. The patients presented with motor, motor and phonic, or only excessive eye blinking tics. Two cases reported had possible drug interactions, so a clear association can only be suspected. The drugs interacting with VPA were ziprasidone and lamotrigine; VPA can increase the levels of ziprasidone/lamotrigine by decreasing their metabolism (Alonso-Navarro et al., 2007; Thome-Souza et al., 2012). The VPA-dose decrease was enough for the achievement of a full recovery in the reports.

The less frequent MD published in the literature in association with VPA was AKT. But, it is noteworthy that this does not represent clinical practice. Friis et al. (1983) assessed 15 individuals using VPA, all of them developed some degree of AKT. Clos (2001) reported the case of a young adult female with bipolar disorder who was prescribed VPA 1,200 mg, and within one month she developed AKT-symptoms, but the diagnosis may be doubtful because the individual was in concomitant use of lithium.

Conclusion

In sum, VPA-associated MD was extensively reported in the literature probably due to availability, costs, and some historical factors of VPA. The most frequent and well described MD was PKN. In descending order of frequency, the following MD related

to VPA were encountered: PKN > MCL > DTN > DKN > Stutter > Tic > AKT. Further studies are warranted to elucidate the occurrence of these MD associated with VPA and its underlying pathophysiology. Future reports need to clearly describe the clinical history of the patient considering a full investigation of other adverse events during their entire life as well as a long-term follow-up. We believe that the knowledge of VPA-associated MD raises the awareness about MD, and especially those drug-induced, which sometimes are challenging in the clinical practice to diagnose and manage.

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Comparison of the Morse Cone Connection with the Internal Hexagon and External Hexagon Connections Based on Microleakage – Review

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Abstract: The gap formed at the abutment-implant interface brings about a bacterial colonization. In addition, a bacterial reservoir can be established within the implant. The build-up of microorganisms around the implant can cause soft tissue infections and bone loss around the implant, which can lead to implant failure. Our literature review aimed to evaluate the infiltration at the implant-abutment interface, comparing the Morse cone connection with the external hexagon and internal hexagon connections. A literature search using the PubMed database was performed on March 24, 2021. The search terms were combinations of "Morse cone" or "Morse taper" with each of the following terms (individually): "microleakage", "leakage", "infiltration", and "penetration". The inclusion criterion was *in vitro* studies comparing the Morse cone with the external hexagon and/or internal hexagon, based on infiltration at the implant-abutment interface. The exclusion criterion was the evaluation of microleakage at the implant-abutment interface after applying a sealant over this region. The search was expanded as needed. There was no limit on the year of publication, and only articles written in English were included. In addition, references cited in included articles were also included in this review when they were appropriate. This literature review concluded that, in most cases, the microleakage in the Morse cone connection was lower when compared with the external hexagon and internal hexagon connections.

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Introduction

In implantology, implant-abutment connections have been used clinically for many decades (Scarano et al., 2016a). Basically, two connections are available: internal and external connections (Goiato et al., 2015). The external connection usually has an external hexagon on the implant platform, and the internal connection can be divided into internal hexagon, internal octagon, and Morse cone, which has been currently widely used (Goiato et al., 2015).

Even when the implant and abutment are connected correctly, a microgap is formed (Pereira et al., 2016); so, there is no implant connection that can provide a complete seal at the implant-abutment interface (Schmitt et al., 2014). In addition, this microgap can increase in size over time due to masticatory loads that can cause micromovements of the prosthesis components (Scarano et al., 2015; Pereira et al., 2016). Regardless of the type of connection, the implant-abutment interface (with a microgap) is present and may be located at different depths of the bone crest (subcrestal, equicrestal or supracrestal), depending on the connection used (Dibart et al., 2005; Ricomini Filho et al., 2010; Verdugo et al., 2014).

The microleakage produced by a microgap between implant and abutment, allows the passage of acids, enzymes, bacteria (Verdugo et al., 2014) (commensal and/or pathogenic bacteria, especially anaerobic or microaerophilic species) (do Nascimento et al., 2012) and/or their metabolic products (Verdugo et al., 2014). Therefore, the microgap formed at the abutment-implant interface becomes a reservoir of microorganisms, and these microorganisms can still infiltrate into the implant (Ricomini Filho et al., 2010; Scarano et al., 2015; Mishra et al., 2017).

Bacterial penetration at the implant-abutment interface can occur under static conditions and during masticatory function (Scarano et al., 2015). The degree of union between abutment and implant, micromovements between them and the applied torque determine different amounts of bacterial movements (from outside to inside and vice and versa – "pump effect") in the different implant connections (Scarano et al., 2015).

Accumulation of microorganisms around the implant due to a microgap, can cause soft tissue infections (Scarano et al., 2015; Mishra et al., 2017) (causing bleeding, swelling and a bad odour) (Verdugo et al., 2014) and bone loss around the implant, which can lead to implant failure (Scarano et al., 2015; Mishra et al., 2017). According to Dibart et al. (2005) this situation occurs because the sustained activation of inflammatory cells promotes the formation and activation of osteoclasts, which can result in alveolar bone loss. Therefore, it is important to ensure the minimum presence of bacteria inside or around the implant-abutment junction (Dibart et al., 2005).

Our literature review is aimed to evaluate the infiltration at the implant-abutment interface, comparing the Morse cone connection with the external hexagon and internal hexagon connections.

Material and Methods

A literature search using the PubMed database was performed on March 24, 2021. The search terms were combinations of "Morse cone" or "Morse taper" with each of the following terms (individually): "microleakage", "leakage", "infiltration", and "penetration". The inclusion criterion was *in vitro* studies comparing the Morse cone with the external hexagon and/or internal hexagon, based on infiltration at the implant-abutment interface. The exclusion criterion was the evaluation of microleakage at the implant-abutment interface after applying a sealant over this region. The search was expanded as needed. There was no limit on the year of publication, and only articles written in English were included. In addition, references cited in included articles were also included in this review when they were appropriate. Twelve articles on the specific purpose of this review were included in this study (Ricomini Filho et al., 2010; do Nascimento et al., 2012, 2015; Jaworski et al., 2012; Tripodi et al., 2012; D'Ercole et al., 2014; Sahin and Ayyildiz, 2014; Verdugo et al., 2014; Scarano et al., 2015; Pereira et al., 2016; da Silva-Neto et al., 2017; de Sousa et al., 2019). The other articles included aimed to contextualize the reader and discuss the review.

Implant connections

External hexagon connection

The external hexagon was the first connection system adopted in modern implantology by Brånemark (Brånemark et al., 1977; Brånemark, 1983; Ceruso et al., 2017) and was subsequently improved (Ceruso et al., 2017). Despite this, this type of connection has 3 disadvantages according to Verdugo et al. (2014):

1) There is little contact length between the restoration and the hexagonal part of the implant head (0.7 mm) (Verdugo et al., 2014).
2) The seating of the secondary component provides a degree of freedom between it and the main component; this allows a degree of rotation between the external hexagon of the platform and the internal hexagon of the restoration component (Verdugo et al., 2014).
3) There is great tension in the screw connection. The screw is essentially the only resistance device in the connection unit, so all the force generated due to the micromovements is released on the screw (Verdugo et al., 2014). As a result, the screw tends to loosen and/or fracture relatively easily (Verdugo et al., 2014).

Internal hexagon connection

The connection of the internal hexagon was developed to improve factors such as stress distribution and contact area between the implant and the secondary component (Verdugo et al., 2014; Ceruso et al., 2017).

According to Verdugo et al. (2014):

In the internal hexagon connection, the hexagon and the screw pass into the implant body and the length of the hexagon increases to 1.2 mm; thus, the

prosthetic component is more stable, even without the fixing screw (Verdugo et al., 2014). The greater the length of contact between the implant and the external component, the lower the tension for the fixation screw, reducing the probability of loosening the screw (Verdugo et al., 2014). The force generated by the micromovements in this type of connection is dissipated to the walls adjacent to the implant hexagon and in a lower degree to the screw (Verdugo et al., 2014). This lower tension on the fixing screw can also be seen in the Morse cone connection (Verdugo et al., 2014).

Morse cone connection

In 1864, this connection was developed by Stephen A. Morse, and since has been used worldwide to connect drilling machines to a removable rotating drill piece (Ranieri et al., 2015; Macedo et al., 2016). The basic principle of this system is "a cone within a cone" (Hernigou et al., 2013; Ranieri et al., 2015). In dentistry, in addition to the mechanical locking of the Morse cone system, a screw retention is added to this system (Vinhas et al., 2020). The taper angle of the Morse cone system can be 8, 11 or 16° (Merz et al., 2000; Khorshidi et al., 2016; Vinhas et al., 2020). It is also worth mentioning that the Morse cone implant accepts different abutment platforms (Macedo et al., 2016).

Review

According to Scarano et al. (2015) several techniques have been used, *in vitro*, to evaluate the sealing ability of the implant-abutment interface, such as, bacteria, bacterial toxins, dyes (toluidine blue and gentian violet), gas, saliva and etc.

Morse cone versus external hexagon

Verdugo et al. (2014) evaluated microleakage (methylene blue) at the implant-abutment interface by comparing the Morse cone (MG InHex®) with the external hexagon (Osseous MG®). Verdugo et al. (2014) observed that after mechanical and thermal cycling (2,000 cycles of 10 k every 0.5 s; and two sessions of 300 cycles in water at 5 °C for 5 s and then at 50 °C for 5 s), regardless of the torque used (hand tightening, 20 N and 30 N [recommended by the manufacturer]), the Morse cone showed significantly less microleakage when compared with external hexagon (the microleakage was observed with an optic microscopy). Furthermore, in this study it was observed that the microgap formed between abutment and Morse cone implant was 2–3 μm, while the microgap formed by the external hexagon connection was 10 μm (Verdugo et al., 2014).

Jaworski et al. (2012) compared the Morse cone connection (10 Ncm of torque – Titamax CM, Neodent®) with the external hexagon connection (32 Ncm of torque – Titamax Ti cortical, Neodent®) based on microleakage (*Escherichia coli*). Both torques were applied according to the manufacturer's recommendations (Jaworski et al., 2012). This comparison was made by a microbiological analysis (Jaworski

et al., 2012). Sixty percent of the samples in the external hexagon group were contaminated and 30% of the samples in the Morse cone group were contaminated. Therefore, Jaworski et al. (2012) concluded that both connections showed leakage, but the Morse cone connection provided a better bacterial seal than the external hexagon connection.

Scarano et al. (2015) compared the leakage of toluidine blue between the external hexagon connection and the Morse cone connection using mechanical cycling of 1×10^6, 3×10^6 and 6×10^6. The abutments were connected to the implants according to the manufacturer's recommendations (Implacil – De Bortoli®) (Scarano et al., 2015). They observed that no significant differences were detected between these connections when the lowest cycling was used (Scarano et al., 2015). However, a difference was observed when the samples were loaded with 3×10^6 and 6×10^6 cycles, with significantly lower toluidine leakage in the Morse cone group (Scarano et al., 2015).

Ricomini Filho et al. (2010) compared these connections: external hexagon with universal post, Morse taper with universal post (MT-1), Morse taper with universal post through bolt, and locking taper with standard abutment (MT-2), based on bacterial infiltration. The torque of 32 Ncm was used for external hexagon and Morse cone with universal post, and the torque of 15 Ncm was used for Morse cone with universal post through bolt (all torques were applied according to the manufacturers) (Ricomini Filho et al., 2010). Samples (implant-abutment) were subjected to a thermal cycling regimen (1,000 cycles of 5 °C and 55 °C) and to mechanical fatigue (1.0 million cycles, 1.0 Hz, 120 N) (Ricomini Filho et al., 2010). The samples were immersed in Tryptic Soy + Yeast Extract broth containing *Streptococcus sanguinis* and incubated at 37 °C and 10% CO_2 for 72 h (Ricomini Filho et al., 2010). Then, an evaluation of the microleakage was performed with and without thermocycling (Ricomini Filho et al., 2010). The external hexagon groups showed 0% bacterial penetration regardless of the application of thermomechanical cycling or not (Ricomini Filho et al., 2010). The Morse cone groups without thermomechanical cycling had a penetration rate of 40% (MT-2) and 60% (MT-1); and the Morse cone groups after thermomechanical cycling had a penetration rate of 50% (MT-2) and 67% (MT-1) (Ricomini Filho et al., 2010).

Pereira et al. (2016) evaluated the removal torque and the biofilm penetration at the implant-abutment interface of the Morse cone and external hexagon after fatigue (50 N at 30 °C and under 500,000 cycles at 1.2 Hz in growth medium containing human saliva for 72 hours). Before mechanical cycling, the Morse cone abutment screws were tightened using a torque of 15 Ncm, while the external hexagon abutments were tightened using a torque of 32 Ncm, according to the manufacturer's recommendation (Neodent®) (Pereira et al., 2016). Pereira et al. (2016) concluded that the optical density of biofilms and the mean CFU (colony-forming unit) value were significantly lower in the Morse cone group than in the external hexagon group. The mean values of removal torque were significantly

lower for both connections (Morse cone and external hexagon) after fatigue (Pereira et al., 2016). The gap sizes before fatigue were 1.7 ± 0.4 μm (Morse cone) and 1.5 ± 0.4 μm (external hexagon), and after fatigue were 3.2 ± 0.8 μm (Morse cone) and 8.1 ± 1.7 μm (external hexagon) – significant increase in both cases (Pereira et al., 2016).

de Sousa et al. (2019) compared the Morse cone connection (20 Ncm) with the external hexagon connection (30 Ncm) based on microleakage. Both torques were applied according to the manufacturer's recommendations (DSP Biomedical®) (de Sousa et al., 2019). Through a microbiological evaluation, de Sousa et al. (2019) found that there was no significant difference in the bacterial growth of *Enterococcus faecalis* or *Candida albicans* or *Enterococcus faecalis* + *Candida albicans* between the Morse cone and external hexagon groups.

Morse cone versus internal hexagon

In the study by Sahin and Ayyildiz (2014), to measure the leakage at implant-abutment interface, a modified fluid filtration method using deionized water with pressure was used. When comparing the Morse cone connection with the internal hexagon connection, both titanium abutments (torque of 25 Ncm – manufacturer's recommendation), it was possible to verify that there was no significant difference regarding the microleakage or loss of torque between them (Sahin and Ayyildiz, 2014). However, when comparing Morse cone (titanium abutment/torque of 25 Ncm – manufacturer's recommendation) with internal hexagon (zirconia abutment/torque of 25 Ncm – manufacturer's recommendation), the internal hexagon connection showed a microleakage and torque loss significantly greater than the Morse cone connection (Sahin and Ayyildiz, 2014). Sahin and Ayyildiz (2014) speculated that microleakage causes the screw to loosen, removing torque values as microleakage increases. In addition, the removal torque values reached up to 91% of the initial tightening torque value (25 Ncm) (Sahin and Ayyildiz, 2014).

D'Ercole et al. (2014) evaluated in a period of 28 days, the microbial leakage of the implant-abutment connection of the Morse cone and internal hexagon systems (Dentoflex®). Ten specimens of Morse cone (group 1) and 10 of internal hexagon (group 2) implants were used (D'Ercole et al., 2014). The inner parts of 5 implants per group were inoculated with *Pseudomonas aeruginosa* and 5 implants per group with *Aggregatibacter actinomycetemcomitans* (D'Ercole et al., 2014). D'Ercole et al. (2014) used a microbiological method to evaluate microleakage. The result showed a high permeability related to bacterial leakage from the internal hexagon connection and the lower infiltration rates – although not significantly – of the Morse cone connection (D'Ercole et al., 2014). Tripodi et al. (2012) carried out a study similar to that carried out by D'Ercole et al. (2014), and Tripodi et al. (2012) obtained results similar to those observed in the study by D'Ercole et al. (2014).

Morse cone versus external and internal hexagon connections

do Nascimento et al. (2012) evaluated the bacterial leakage from human saliva to the internal part of the implants along the implant-abutment interface under loaded (500,000 cycles at 120 N) and unloaded conditions using DNA Checkerboard. The evaluated connections (SIN, Sistema de Implante Nacional®) were: 1) external hexagon, 2) internal hexagon, and 3) Morse cone (do Nascimento et al., 2012). In all connections, the titanium abutment screws received a torque of 20 Ncm and the crown received 10 Ncm, as recommended by the manufacturer (do Nascimento et al., 2012). After fatigue, the external hexagon and internal hexagon connections showed significantly higher bacterial counts than the Morse cone connection (do Nascimento et al., 2012). When the specimens in the control groups (without loading) were compared with the specimens with loading, the external hexagon and internal hexagon connections of the loaded groups showed significantly higher bacterial counts than their unloaded counterparts (do Nascimento et al., 2012). However, the bacterial counts were not significantly different for the Morse cone connection between the control group (without loading) and the group with loading (do Nascimento et al., 2012).

do Nascimento et al. (2015) evaluated the bacterial infiltration (DNA Checkerboard method) of prostheses supported by external hexagon or Morse cone implants (Signo Vinces®). do Nascimento et al. (2015) performed mechanical cycling on the specimens (150 Ncm during 500,000 cycles at 1.8 Hz). Before loading, the titanium abutment screws were tightened to 20 Ncm and the prosthesis screws to 10 Ncm in all connections (manufacturer's recommendation) (do Nascimento et al., 2015). Twenty-one bacterial species, including periodontal pathogens and *Candida albicans*, were found colonizing the internal surfaces of the external hexagon implants after loading. No microorganisms were detected in the internal parts of the Morse cone implants after loading (do Nascimento et al., 2015). In general, the external hexagon and Morse cone implants showed similar values of marginal gap before and after loading (do Nascimento et al., 2015). The mean values of microgap vertical recorded for all groups were low, ranging from 12 to 25 μm (do Nascimento et al., 2015).

da Silva-Neto et al. (2017) compared the Morse cone with external hexagon and internal hexagon based on microleakage. The application of the torques followed the manufacturer's instructions (Neodent®) (da Silva-Neto et al., 2017). Using toluidine blue and mechanical cycling (300,000 cycles, 50 N, 1.2 Hz), da Silva-Neto et al. (2017) found that the Morse cone connection was significantly more effective in preventing microleakage after fatigue than the internal hexagon connection. It is worth mentioning that in this same study, after 300,000 cycles, there was no significant difference based on microleakage between the Morse cone and external hexagon; however lower mean microleakage values were observed for the Morse cone connection (da Silva-Neto et al., 2017).

Discussion

In scientific research, different methodologies can generate different results. Despite this, most of the studies evaluated in this review showed that less microleakage was related to the Morse cone connection when compared with the external hexagon and internal hexagon connections.

Few studies included in this review reported the microgap sizes of the evaluated connections (Verdugo et al., 2014; do Nascimento et al., 2015; Pereira et al., 2016). Regardless of the type of connection, the microgap sizes shown in this review ranged from 1.5 to 25 μm (Verdugo et al., 2014; do Nascimento et al., 2015; Pereira et al., 2016) and, therefore, these microgap sizes are within the clinically acceptable range (based on implant-abutment interface, an acceptable microgap size should not exceed 120 μm) (do Nascimento et al., 2015). It is worth mentioning that the mean size of microorganism species in the oral microbiota ranges from 1.1 to 1.5 μm in diameter and 2 to 6 μm in length (do Nascimento et al., 2015). Therefore, bacterial microleakage can be expected in any connection.

Verdugo et al. (2014) and Pereira et al. (2016) reported smaller microgap sizes for the Morse cone connection when compared with external hexagon, which may explain less microleakage related to cone Morse connection in most cases of this review. Scarano et al. (2016b, c) analysed with 3-dimensional X-ray microtomography, the microscopic space that exists between the implant body and abutment, comparing the internal hexagon with Morse cone. It was observed that in all cases for the Morse cone connection there was no detectable separation between implant and abutment. However, for the internal hexagon connection, numerous microgaps and voids were present (Scarano et al., 2016b, c). Thus, the Morse cone connection appears to be more advantageous than the external hexagon and internal hexagon connections.

The Morse cone connection can resist torque loss more significantly after fatigue than the external hexagon connection (Park et al., 2010). Torque loss can significantly facilitate microleakage (Verdugo et al., 2014), and it is speculated that microleakage can reduce torque values (Sahin and Ayyildiz, 2014). Therefore, resisting the loss of torque is very important for the success of the treatment and, therefore, the Morse cone connection can be more advantageous than the external hexagon connection.

Due to the better results of the Morse cone shown in this review (less microleakage), this connection is possibly less likely to generate inflammation of peri-implant tissues (Verdugo et al., 2014). In addition, the design of the Morse cone generates a separation between the microgap and the marginal bone (Weng et al., 2008; Ranieri et al., 2015; Macedo et al., 2016). Therefore, in theory, a microleakage would occur at a greater distance from the peri-implant tissues (when compared with internal hexagon or hexagon external) (Weng et al., 2008; Ranieri et al., 2015); and this would possibly help to prevent infections of the peri-implant tissues.

Conclusion

This literature review concluded that, in most cases, the microleakage in the Morse cone connection was lower when compared with the external hexagon and internal hexagon connections.

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Pelvic Floor Muscles Contribution in Surgical Outcome of Children with High-type Anorectal Malformations

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Abstract: As a consequence of high-type anorectal malformations (ARMs) pathogenesis, the pelvic floor muscles remain severely underdeveloped or hypoplastic, the rectal pouch is located at the level or above the puborectalis sling, and the bowel terminates outside the sphincter muscle complex support. For children with high-type ARMs the ultimate objective of therapy is mainly to grow up having bowel continence function that is compatible with a good quality of life, and the final prognosis depends significantly on the grade of development of pelvic floor muscles and the successful entering of the anorectum fully within the support of the external anal sphincter due to intraoperative conservation of the puborectalis sling. Pelvic magnetic resonance imaging (MRI) has recently become the preferred imaging study for prediction of functional outcomes, since it can define the anatomy and evaluate the development of the sphincteric muscles before and after surgical correction. Based on recent literature and our clinical experience, we will discuss the relevance of pelvic floor muscles MRI to the clinical outcome of children with high type ARMs.

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Introduction

Anorectal malformations (ARMs) are among the most frequent congenital anomalies in paediatric surgery (Gangopadhyay and Pandey, 2015). They comprise a wide spectrum of anatomical presentations and associated anomalies involving the anorectal and urogenital system, sacral spine, and perineal musculature (Levitt and Peña, 2007; Bhatnagar, 2015a; Wood and Levitt, 2018). ARMs are divided into low, intermediate, and high types depending on whether the rectal pouch is located below, at the level, and above the pubococcygeal line (PC) and the ischiadic point (IP) respectively (Alamo et al., 2013). We used the older and rather obsolete classification of the three levels herein, to outline the topographic variations according to the level of the anomaly, implicating rather a more simplified and iconic approach for a radiological view of point, instead of the modern, widespread among paediatric surgeons, clinically-oriented and outcome-associated classification of Pena (van der Steeg et al., 2015), thoroughly discussed at the meeting of Krickenbeck, Germany (Holschneider et al., 2005). There is a ventral displacement of the anal canal which opens either at the perineum or forms a fistula to the urogenital tract (Bhatnagar, 2015a), with the fistulous bowel terminating outside the support of the external anal sphincter (Kyrklund et al., 2017). At the same time, the pelvic floor muscles present varying degrees of hypoplasia ranging from normal musculature to absent muscle complex, depending on the severity of the lesion.

The pelvic floor is composed of a funnel-shaped sling of muscles with supporting and constricting functions, extending from the symphysis pubis to the coccyx, and from one lateral sidewall of the pelvic cavity to the other (Raizada and Mittal, 2008). The subcutaneous portion of the external anal sphincter (EAS) is located caudal to the internal anal sphincter (IAS), the superficial portion surrounding the distal part of the IAS, and the deep portion merging imperceptibly with the puborectalis muscle (Raizada and Mittal, 2008). In fact, EAS is not entirely a circular muscle, but is attached to the puboperineal muscle on either side (Ayoub, 1979). The puborectalis muscle is located between the superficial layers of the anal sphincters and the deep muscle layers of the levator ani muscle (Raizada and Mittal, 2008). Levator prostate or sphincter vaginae, ischiococcygeus, iliococcygeus and pubococcygeus, constitute the levator ani muscle, with some pubococcygeus fibers looped around the rectum forming the puborectalis as the most inferior part of the levator ani muscle group (Raizada and Mittal, 2008; Alamo et al., 2013). The term of a puborectal muscle sling isn't accepted by many pediatric surgeons performing this type of pediatric surgery. However, the future studies concerning this part of anorectal sphincter mechanism may confirm its existence.

Magnetic resonance imaging (MRI) has a leading role both before and after surgical correction of a high type ARM. Among others, MRI provides detailed structural information on the pelvic musculature anatomy and development of children with high type ARMs. Ultimate objective of management is mainly the

outcome of a bowel within the best achievable results in anatomy and continence (Alamo et al., 2013), as high-type anomalies are characterized by major structural deficits, non-compatible with a fair quality of life. This review aims to outline essential points regarding the functional anatomy of the pelvic muscles in high-type ARMs.

Evolution of surgical treatment in relation to the pelvic floor muscles

Stephens in 1953, the first paediatric surgeon who studied the anatomy of the pelvis in children with ARMs, concluded that the puborectalis sling is the key part of the sphincter mechanism to achieve postoperative bowel control (Zaiem and Zaiem, 2017). The pull-through techniques were considered as blind techniques as they did not ensure accurate placement of the rectum at the center of the muscle complex. De Vries and Pena in 1982 introduced the posterior sagittal anorectoplasty (PSARP) technique, which ensured the placement of the rectum inside the sphincter mechanism (Zaiem and Zaiem, 2017). It was learned from the procedure that there is a strong funnel-like muscle structure that forms the sphincter mechanism, with its upper portion of horizontal muscle fibers referred as the levator muscle, and its lower portion (named by Pena as the muscle complex) consisted mainly of vertical fibers running parallel to the rectum (Zaiem and Zaiem, 2017). PSARP also underlined the role of the muscle complex as a functional prominent structure (Zaiem and Zaiem, 2017; Patel et al., 2018). Therefore, adequate placement of the neorectum not only through the puborectalis sling but also within the EAS is necessary for an adequate functional outcome (Bhuynan et al., 2015). In an attempt to give the muscle complex its due respect by keeping it intact and restored around the new pulled rectum, sphincter saving anorectoplasty (SSARP) and muscle complex saving posterior sagittal anorectoplasty (MCS-PSARP), have been described (Bhuynan et al., 2015).

The later introduced laparoscopically assisted anorectal pull-through (LAARP) technique for the high-type ARMs, aiming to reduce the amount of posterior dissection of the sphincter mechanism required for the accurate placement of the neorectum into the muscle complex under laparoscopic vision (Georgeson et al., 2000). The avoidance of division of the vertical muscle complex owed to the longitudinal cutting of the sphincter muscle complex with the PSARP technique, resulted in the minor disturbance of the muscle innervations (Bhuynan et al., 2015). The laparoscopic approach resulted in better sphincter symmetry, and lesser irregularity and perirectal fibrosis compared to PSARP (Bhatnagar, 2015c). A limitation of the method is that the narrow path of the vertical muscle fibers between the pelvic floor and the perineal parasagittal muscle fibers cannot be visualized (Raschbaum et al., 2010). This raises the likelihood of deviation from the course of the central portion of the vertical muscle complex while performing pull-through of the rectal segment to the perineum (Raschbaum et al., 2010).

Preoperative pelvic floor muscle MRI

MRI has a defined role in the imaging protocol of ARMs (Madhusmita et al., 2018), because of its lack of ionizing radiation, excellent intrinsic contrast resolution, and multiplanar imaging capabilities (Bhatnagar, 2015c). Disadvantages comprise an occasional lack of expertise or access to the technique, a relative high cost, and the need for sedation (in neonates and young infants the examination must be done with general anaesthesia or by the way of a "feed and wrap" i.e., sleeping after eating) (Podberesky et al., 2013). Pelvic MRI has recently become the preferred imaging study for defining the anatomy and evaluating the size, the morphology, and the grade of development of the sphincteric muscles before surgical correction (Weinstein et al., 2009; Alamo et al., 2013; Bhatnagar, 2015b). Functional prognosis of high type ARM can be predicted, since children with an underdeveloped sphincter muscle complex are likely to be incontinent (Madhusmita et al., 2018).

In neonates and infants with ARMs a pelvic MRI protocol should include T1 and T2 weighted images in coronal, sagittal, and axial (transverse) planes. The anatomic characteristics of the sphincter muscle complex are clearly demonstrated by routine T1 and T2 weighted images but are depicted more affluently on T2 (Tang et al., 2006). Restrained fat signal can be applied for better muscle visualization (Tang et al., 2006). No anatomic distinction between the individual muscles of the levator ani or the EAS can be routinely detected by MRI, nevertheless their distinction is of no clinical value in the setting of ARM correction (Podberesky et al., 2013). The pubococcygeal plane corresponds to the attachment level of the levator ani muscle to the pelvic wall, extends from the upper border of the os pubis to the os coccyx, and includes the prostate in males or the cervix in females and the rectum (Alamo et al., 2013). In the axial images of this plane, the puborectalis muscle appears as a triangular band surrounding the rectum posterolaterally (Alamo et al., 2013; Madhusmita et al., 2018). A well-developed levator ani is seen clearly on coronal images as a sling-like structure supporting the rectal ampulla (Madhusmita et al., 2018). The levator ani muscle fibers are intraoperatively recognized as vertical fibers attached to the sacrum and to the rectal wall. The ischial plane following the line joining the lowest points of the ischial tuberosities represents the deepest point of the funnel of the levator ani muscles (Alamo et al., 2013). The oval shaped external anal sphincter posteriorly can be clearly observed in this plane and the deep portion of the EAS can be distinguished in axial images from the cranially located directly adjacent puborectalis muscle, appearing to overlap the puborectalis muscle bundles (Alamo et al., 2013). The EAS is seen as a posterior curved band-like structure in sagittal images, with fibers extending in the parasagittal images, and as an oval structure symmetrically surrounding the anal canal in the axial images (Madhusmita et al., 2018). In the midsagittal plane, the EAS encircles the anal canal both anteriorly and posteriorly, with the lower anterior part extending ventrally and the posterior extending to and connecting with the coccyx, caudally to the puborectalis muscle (Alamo et al., 2013).

MRI images are evaluated for the subjective developmental state of the sphincter muscle complex, in particular the length, width, and thickness of the puborectalis and EAS muscles (Tang et al., 2006; Madhusmita et al., 2018). Although some studies used objective measurements of the sphincteric and levator sling muscles to describe their overall quality on MRI, a subjective good, moderate, or poor assessment is typically adequate (Podberesky et al., 2013; Madhusmita et al., 2018). Deviation from the defined as normal appearance is evaluated as fair or moderate. When the muscle fibers are poorly visualized, they quality is graded as poor (Madhusmita et al., 2018). The higher the ARM, the poorer the sphincter muscle complex development is seen in MRI (Kyrklund et al., 2017; Madhusmita et al., 2018). In children with low ARMs, the well-developed sphincteric muscles usually demonstrate normal or almost-normal size and morphology at MRI (Alamo et al., 2013). Moreover, the rectum is usually located within most of the sphincters, except an anteriorly mislocated lower part in some cases (Alamo et al., 2013). In children with high type ARM, the underdeveloped sphincteric muscles are frequently asymmetric and highly hypoplastic (Alamo et al., 2013). Most females with a cloacal anomaly have hypoplastic and underdeveloped levator ani muscle and EAS, and these with a longer common canal present highly to extremely hypoplastic and underdeveloped levator ani muscle, and almost unrecognizable EAS (Alamo et al., 2013).

There are MRI measurement indexes that can be taken as quantitative criterion for poor developmental state of pelvic floor muscles (Madhusmita et al., 2018). These indexes could be used as clinical predictive factors for surgical outcome of children with high type anorectal malformations. The relative width of the puborectalis muscle (RWPR) and EAS (RWEAS) on a transverse plane, are defined as the ratio of the total width of muscle (as the sum of both left and right muscle width of the rectum or anal canal) and the half distance of ischial tuberosities (Madhusmita et al., 2018). The relative length of puborectalis muscle (RLPR) and EAS (RLEAS) on a sagittal plane, are defined as the ratio of the length of muscle and the length of the pubococcygeal line (Madhusmita et al., 2018). When PRWR is < 0.18 and EASWR < 0.15, 71% of the patients with ARMs suffer from anal incontinence postoperatively (Madhusmita et al., 2018). When PRWR is > 0.18 and EASWR > 0.15, 91% of the patients with ARM have good continence, and the poor continence in the remaining patients is mainly due to constipation (Madhusmita et al., 2018). All paediatric surgeons who perform ARMs corrective surgery are familiar with the widespread index known as sacral ratio, based on the sacral length with anatomic pelvic bone landmarks, and measured with lateral pelvic radiographs. Values below 0.4 have a predictive value of poor functional outcome. Krois et al. (2021) in a retrospective cohort study, demonstrated recently that the sacral ratio can be calculated on MRI with a good reliability, also providing the advantage of less exposure to ionizing radiation.

Postoperative pelvic floor muscle MRI

Except the musculature developmental status, appropriate placement of the rectal pull-through within the levator muscle, which is the most important factor of continence, and the EAS, is critical for optimal postoperative bowel continence (Bhatnagar, 2015b; Bhuynan et al., 2015). MRI is the optimal method for the evaluation of postoperative fecal incontinence, the study of complications, and the design of management in cases of consideration of potential reoperation (Podberesky et al., 2013; Bhatnagar, 2015c; Madhusmita et al., 2018). MRI evaluation includes the developmental quality and the postoperative shape of the striated muscle complex, the positioning of the neorectum in relation to the muscles, the anorectal angle, the peritoneal fat herniation, and the postoperative muscle scarring, outlining as a main task to show a mislocated rectum and the damaged sphincteric muscles (Podberesky et al., 2013; Yong et al., 2013; Raman et al., 2015).

T1 and fast or turbo spin-echo T2 weighted sequences are applied in the axial/sagittal/coronal planes without fat saturation, with the surgeon interested and focused in the midsagittal sections because it is the plane used for the operative approach (Eltomey et al., 2008). Anterior misplacement of the neorectum in the EAS, and lateral misplacement of the neorectum in the puborectalis muscle, are the most common surgical errors observed (Madhusmita et al., 2018). The sphincter muscle complex is best seen in the axial images at the level of the symphysis pubis and below (Eltomey et al., 2008; Raman et al., 2015). Axial and coronal images show better a side to side displacement of the bowel, while sagittal images help in the assessment of an anteroposterior displacement of the bowel in relation to the sphincter (Eltomey et al., 2008; Raman et al., 2015).

The measurement of the thickness/continuity/regularity of the pelvic muscles is actually a subjective assessment, based on the internal symmetry from side to side and the comparison with normal pelvic musculature in similar aged, healthy patients (Podberesky et al., 2013; Farghaly et al., 2018). Development of the striated muscles is defined as good if the muscle has a regular shape and normal thickness, intermediate dysgenesis if the muscle is intact with reduced thickness, and poor dysgenesis if the muscle is disrupted or deformed (Yong et al., 2013). In some cases, the bowel is properly positioned but mesenteric fat that is inadvertently pulled with bowel through the sphincter during the initial repair interferes with the continence mechanism (Eltomey et al., 2008). Peritoneal fat herniation occurs in cases of colonic pull-through, if a rectum must be excised if too short or ischemic, and replaced by colon. Fat herniation weakens the rectal fullness sensation and voluntary muscles stretching ability, by damaging the integrity of the muscle complex (Yong et al., 2013).

Extensive postoperative muscle scarring may cause defecatory dysfunction, and due to its stiff appearance and lower T2 signal can be easily differentiated from normal muscle tissue (Yong et al., 2013).

Children with a lower obtuse anorectal angle have a better clinical outcome (Desai et al., 2018). The normal angle is < 90° and any angle > 100° is considered abnormal (Farghaly et al., 2018). The changes in the anorectal angle are predominantly related to the development status of puborectalis muscle (Raizada and Mittal, 2008; Wahab et al., 2017).

Descending perineum syndrome may be caused by damage to the muscles and ligaments of the pelvic floor during the operation (Levin, 2018), in combination with coexisting hypoplastic muscle weakness. The descent of the pelvic floor is mostly related to the pubo/ileo/ischio-coccygeous muscles (Raizada and Mittal, 2008). A rectal descent (descent of the anorectal junction below the pubococcygeal line) is classified as mild (2–4 cm), moderate (4–6 cm), or severe (>6 cm) (Yong et al., 2013).

Children with abnormally located neorectum and/or increased anorectal angle and/or peritoneal fat herniation and/or pelvic floor dysfunction and/or extensive scarring often require further surgery (Yong et al., 2013). In children with isolated maldevelopment of striated muscle complex, conservative treatment should be offered to relieve symptoms (Yong et al., 2013).

What about the internal anal sphincter?

The practical significance of the intrinsic circular muscle located at the rectal end, known as internal anal sphincter (IAS) has remained controversial in high type anomalies in the relevant literature (Mirshemirani et al., 2009). A functional IAS in anorectal manometry, as indicated by a positive rectoanal inhibitory reflex, has been associated with improved functional outcomes in children who have high or intermediate ARMs by certain researchers (Husberg et al., 1997; Kyrklund et al., 2017). Animal models of ARMs have shown dysplastic IAS with variability in shape and size (Cleeve et al., 2011). Studies reported that the intrinsic muscle layers or the muscle in total are characteristically abnormal and hypoplastic in neonates with high type ARMs (Meier-Ruge and Holschneider, 2000). Functional studies in children with different levels of ARM demonstrated an intact postoperative recto-anal inhibitory reflex (Cleeve et al., 2011). A smooth muscle layer can also be seen close to the opening of the fistula from the rectum to the urogenital tract (Husberg et al., 1997). In addition, electrical field stimulation on smooth muscle strips from the caudal part of the rectal pouch have shown the same characteristics as that of IAS (Husberg et al., 1997; Desai et al., 2018). It was speculated it maybe of functional significance (Cleeve et al., 2011). If the fistula was preserved and transposed to the normal position of the anal canal, an IAS function was anticipated in most cases (Mirshemirani et al., 2009). These findings favoured the conservation of the distal part of the fistula in severe ARMs (Kyrklund et al., 2017). It was stated that the most caudal rectal internal circular muscle fibers have the potential to degenerate and develop as IAS after been transplanted, even though they are histologically loose and hypoplastic at the definitive surgery and contribute to the improvement of passive

continence in the late postoperative period (Mirshemirani et al., 2009). We believe that IAS does not play an important role, particularly in the so-called high ARMs. Occasionally, its resection together with a fistula and the adjacent portion of the rectum if aganglionic is mandatory.

MRI allowed the possibility to show the anatomic evidences of an IAS-like structure encircling the anal canal in all patients operated with PSARP for high or intermediate ARMs, independently of the severity of the malformation or the postoperative physiological IAS function (Husberg et al., 1997). These fibers are notably more irregular, with variations in thickness in different directions or levels in the anal canal and present a larger surface area than those of normal children (Husberg et al., 1997).

What is new with pelvic floor muscles MRI in the anorectal malformations?

MRI guided LAARP is now available. An MRI compatible needle is used to penetrate the perineal skin at the central site of the parasagittal muscle contraction as determined by direct muscle stimulation (Raschbaum et al., 2010). Serial scans are obtained in axial, coronal, and sagittal planes as the needle is advanced cephalad to remain within the central portion of the vertical muscle complex until the peritoneal floor is penetrated (Raschbaum et al., 2010). This method promises better outcome, because of accurate placement of the rectal pull-through within the muscles and less muscle surgical trauma.

In recent years dynamic MRI has been used in older cooperative children and adolescents with pelvic floor dysfunction and fecal incontinence (Boemers et al., 2006; Yong et al., 2013; Wahab et al., 2017; Levin, 2018). Minimal function (movement) of pelvic floor during evacuation attempt, and poor elevation or asymmetrical movement of the levator ani during squeeze can be identified postoperatively (Boemers et al., 2006).

Conclusion

Pelvic floor muscle MRI is considered as required for high-type ARM assessment. The introduction of preoperative and postoperative MRI in the evaluation and management of children with high type ARMs resulted in more detailed anatomical knowledge and better understanding of the clinical importance of the striated muscle complex of the pelvic floor. We may at last identify details of the preoperative congenital deviations and the postoperative outcomes under a powerful light instead of speculating in the shadows. Operative guidance is a significant novelty in the use of pelvic floor muscle MRI for the surgical correction of ARMs.

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Does Systemic Arterial Hypertension Change the Function of the Stomatognathic System?

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Abstract: The aim of this study was to evaluate the stomatognathic system of individuals with controlled systemic hypertension through comparison with a disease-free control group. Seventy individuals (44 female and 26 male) were divided into two groups: a controlled systemic hypertension (n=35) and a disease-free control (n=35). The individuals were evaluated on the basis of masticatory cycle efficiency of the value of the ensemble-averaged integrated linear envelope to the electromyographic signal of the masseter and temporalis muscles in the habitual (peanuts and raisins) and non-habitual chewing (Parafilm M); molar bite force (right and left) and ultrasound images from the bilateral masseter and temporal muscles at rest and maximum voluntary contraction. The data obtained were tabulated and submitted to statistical analysis ($p<0.05$). There was a significant difference between

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groups in the habitual (peanuts and raisins) and non-habitual (Parafilm M) chewing with reduced muscle activity to controlled systemic hypertension group. Muscle thickness occurred significant difference between groups at rest and maximum voluntary contraction of the temporalis muscles. There was no significant difference between groups in maximum molar bite force. The present study findings indicate that the controlled systemic hypertension promotes functional changes of the masticatory system, especially with respect to its masticatory efficiency and muscle thickness.

Introduction

Diseases such as systemic arterial hypertension are associated with vascular changes mediated by reactive oxygen species that promote changes in vascular tonus, remodelling, and inflammation (Vaka et al., 2018; Xu et al., 2020; Yang et al., 2020). In addition to changes to the vascular system, structural changes of organs such as the kidneys, lungs and heart are also directly related to increased generation of reactive oxygen species in hypertension and cardiac hypertrophy (Garg et al., 2020).

The prevalence of systemic arterial hypertension in the world's population is 26% in adults, and it is believed to reach 40% in certain regions. It is estimated that this percentage will further increase by 2025 to reach 29% (Kearney et al., 2005; Lacruz et al., 2015).

In the early stages of systemic arterial hypertension, functional factors predominate in elevating peripheral resistance; in chronic hypertension, structural factors such as vascular remodelling and hypertrophy contribute (Lewington et al., 2002). Muscle function in hypertensive individuals may be affected due to the increase in peripheral vascular resistance and inadequate regulation of oxygen supply to the skeletal muscle (Nyberg et al., 2015). Whereas the inducing oxidative stress in muscle during hypertension brings an increase of angiotensin II, muscle contraction stimulates thin fibers muscle afferents and evokes reflex sympathetic excitation (Koba et al., 2013).

The problems arising in the presence of systemic arterial hypertension involve the striated skeletal muscle system, with impairment of muscle function. Therefore, changes in the activity of the stomatognathic system may also occur. However, no work on systemic arterial hypertension has been found relating to hypertension and mastication muscles. This fact highlights the need to analyse the relationship between the stomatognathic system and chronic degenerative diseases. This study is aimed at the analysis of chewing cycles, muscle thickness, and bite force in individuals with hypertension.

The main hypothesis of the study was that systemic arterial hypertension disease promoted functional changes in the stomatognathic system. This research guides and directs health professionals to perform more cautious oral rehabilitation treatments with the function of promoting clinical success with better quality of life for patients with arterial hypertension.

Material and Methods

Study design and sample

This observational study was approved by the ethics committee (process # 2011.1.1162.58.9). The individuals were informed about the objectives and stages of the study and gave written informed consent.

The post hoc sample size was calculated considering a level of $\alpha = 0.05$, a power of 100% for the main outcome Parafilm M chewing of the right master muscle (mean [standard deviation]: medically controlled hypertension group, 1.20 [0.13] and control group, 1.58 [0.11]) and effect size of 3.15. The minimal sample size obtained was 70 volunteers (35 for each group). The sample size was calculated with the G*Power 3.1.9.2 software.

From an initial cohort of 60 individuals medically controlled hypertension, both genders, with complete teeth and properly rehabilitated through fixed prosthesis of up to one element, 35 were selected for medically controlled hypertension group (mean [standard deviation], 49.2 [1.6] years) on the basis of the eligibility screening criteria.

Sample selection and criteria for inclusion and exclusion were determined by clinical examination and medical history. As inclusion criteria for World Health Organization, individuals should have medical diagnosis of hypertension, were treated, and controlled, and already make use of antihypertensive drugs for at least one year. All individuals should present at least 24 teeth, with at least a molar occlusion in each dental hemi-arch. Individuals with disorders affecting craniofacial growth, a history of epileptic seizures, with removable dental prostheses, and/or using medication or undergoing treatment that could directly or indirectly interfere with muscle activity (antihistamines, sedatives, homeopathy or other depressants of the central nervous system), presence of parafunctional habits, and possible symptoms of temporomandibular disorders (Research Diagnostic Criteria for Temporomandibular Disorders), were excluded from participation in the study.

The disease-free control group (mean [standard deviation], 49.1 [1.6] years) was comprised of individuals who were age, weight, and height matched with the individuals in medically controlled hypertension group (Table 1). Each group had 22 women and 13 men.

Table 1 – Mean, standard error mean (±) and statistical significance ($p<0.05$) of demographic data in hypertensive and disease-free control groups

Groups	Age	Weight	Height
Hypertensive	49.2 ± 1.6	77.06 ± 2.78	1.65 ± 0.01
Control	49.1 ± 1.6	72.81 ± 1.77	1.68 ± 0.01
P-value	0.20	0.21	0.96

The tests applied in this study were performed at a single time by one calibrated investigator who participated in full in the collection of all data.

Masticatory efficiency

The electromyographic signals of the masticatory cycles were collected using the Myosystem BR-1 portable electromyograph (Data Hominis, Uberlandia, Minas Gerais, Brazil), with analog bandpass filters for a cut-off frequency of 10–1,000 Hz, scanning for sample frequency of 4 kHz, and 12-bit resolution. Silver/silver chloride bipolar surface electrodes (Data Hominis Ltd., Model DHT-EASD) with diameter and inter-electrode distance of 10 mm were used. The maximum voluntary contraction manoeuvre was performed for the correct positioning of the electrodes (Hermens et al., 2000).

During the collection of electromyographic data, the individuals remained seated upright, with the soles of their feet resting on the ground, their arms resting on their legs, and head erect with the plane of the head parallel to the ground.

In the analysis of the muscle activity during chewing, the dynamics of habitual and non-habitual chewing was determined using the linear envelopment of the electromyographic signals of the masseter and temporalis muscles (Siéssere et al., 2009; da Silva et al., 2019). Non-habitual chewing is a movement with dynamic records and short excursion during mouth opening (articulator type), which are required to reduce the effects of the change of length and muscle (De Luca, 1997).

The electromyographic signals were obtained during the habitual chewing of 5 g of peanuts (hard food) and 5 g of raisins (soft food). Non-habitual chewing was recorded with Parafilm M. Soft and hard food were selected from the same batch, separated in units, and stored in a plastic recipient. This material was kept in a cool and airy place.

At the beginning of the masticatory process, the initial cycles showed a variation in the pattern of the mandibular movement. Therefore, to calculate the results obtained from the integral of the linear envelope of the masticatory cycles, the initial masticatory cycles were eliminated while the central cycles of the electromyographic were maintained. Three initial masticatory cycles were excluded since, in the initial phase of the masticatory process, the first cycles vary considerably during mandibular movement (Palinkas et al., 2019; Righetti et al., 2020).

Muscle thickness

The portable ultrasound device Titan (SonoSite Inc., Bothell, WA, USA) was used for analysis of muscle thickness during the clinical conditions of at rest and dental clenching in maximum voluntary contraction. During collection, the linear transducer was placed transversely in the direction of the fibers of the belly of the masseter muscle, approximately 1.5 cm to 2.0 cm above the angle of the mandible toward the zygomatic arch. For the temporal muscle, the linear transducer was positioned in the region of the temporal fossa, about 1.0 cm to 1.5 cm behind and above the lateral

palpebral commissures on both sides. Three measurements were performed on each muscle, separately (right masseter, left masseter, right temporal and left temporal), with the mean value being considered the muscle thickness (Palinkas et al., 2010; Donizetti et al., 2019).

Bite force

To obtain bite force, an IDDK (Kratos, Cotia, São Paulo, Brazil) model digital dynamometer was used, with a capacity of up to 1,000 N force, adapted to the oral cavity. The operator can choose the scale to be in Newtons. The measures were taken in the first permanent molars (right and left). Bite force was recorded in newton, each subject was asked to bite the device three times with maximum effort, with a 2-min rest between trials. The highest value among three trials was considered the subject's (Palinkas et al., 2010; Verma et al., 2017).

Statistical analysis

After obtaining the masticatory efficiency, muscle thickness and bite force data, a normality test was run, and the data were considered normally distributed. The data were statistically analysed (Statistical Package for the Social Sciences Version 22.0 for Windows, IBM Inc., Chicago, IL, USA). The results were obtained using descriptive analysis for each variable. The values were compared using the student's *t*-test for independent samples, considering a confidence level of 95% ($p<0.05$).

Results

Table 2 shows the data of muscle activity during habitual (peanuts and raisins) and non-habitual chewing (Parafilm M) of masseter and temporalis muscles, thar were

Table 2 – Mean, standard error mean (±) and statistical significance (p<0.05)* of integral of linear envelope in chewing, for each muscle evaluated, in the hypertensive group (HG) and disease-free control (CG)

Chewing	Muscles	HG	CG	P-value
Parafilm M	right masseter	1.20 ± 0.13	1.58 ± 0.11	0.03*
	left masseter	1.03 ± 0.12	1.66 ± 0.13	0.01*
	right temporal	0.94 ± 0.09	1.38 ± 0.09	0.00*
	left temporal	1.11 ± 0.12	1.42 ± 0.09	0.05*
Raisins	right masseter	0.93 ± 0.10	1.47 ± 0.32	0.00*
	left masseter	0.88 ± 0.08	1.21 ± 0.10	0.00*
	right temporal	0.88 ± 0.09	1.06 ± 0.08	0.05*
	left temporal	0.90 ± 0.09	1.18 ± 0.08	0.01*
Peanuts	right masseter	1.25 ± 0.09	1.71 ± 0.12	0.11
	left masseter	1.11 ± 0.09	1.68 ± 0.10	0.01*
	right temporal	1.19 ± 0.12	1.47 ± 0.09	0.16
	left temporal	1.23 ± 0.13	1.82 ± 0.14	0.03*

Table 3 – Mean, standard error mean (±), and statistical significance (p<0.05)* of muscle thickness (mm) in the clinical condition of at rest and dental clenching, in the hypertensive group (HG) and disease-free control (CG)

Clinical conditions and muscles		HG	CG	P-value
Rest	Right masseter	1.06 ± 0.03	1.00 ± 0.02	0.21
	Left masseter	1.05 ± 0.03	1.01 ± 0.02	0.37
	Right temporal	0.40 ± 0.01	0.64 ± 0.02	0.00*
	Left temporal	0.39 ± 0.02	0.63 ± 0.02	0.00*
Dental clenching	Right masseter	1.34 ± 0.04	1.27 ± 0.03	0.25
	Left masseter	1.33 ± 0.04	1.28 ± 0.03	0.40
	Right temporal	0.51 ± 0.02	0.72 ± 0.02	0.00*
	Left temporal	0.50 ± 0.02	0.71 ± 0.02	0.00*

Table 4 – Mean, standard error mean (±) and statistical significance (p<0.05)* of the bite force of the right and left side molar regions in the hypertensive group (HG) and disease-free control (CG)

Force	HG	CG	P-value
Right molar	303.51 ± 28.43	260.46 ± 26.77	0.28
Left molar	321.85 ± 27.94	280.27 ± 33.04	0.33

analysed using the integral of linear envelope. The muscle activity with chewing decreased for hypertension group in hard food and soft food, with a significant difference ($p<0.05$); with exception for the masseter and temporal right muscles during chewing with peanuts where no difference was found.

The average thickness of the muscle at rest and at the maximum voluntary contraction in temporalis muscles (left and right) were lower in the hypertension group ($p<0.05$) (Table 3).

The maximum molar bite force values as shown in Table 4. There were no significant differences ($p<0.05$) between both groups.

Discussion

The purpose of this study was to determine the morphological and functional changes in individuals with medicated and controlled hypertension by using methodologies for measuring masticatory efficiency, muscle thickness and molar bite force.

The muscle activity during chewing showed a decrease of electromyographic activity in all muscles analysed for the hypertensive group. Chewing is a complex and dynamic process. The basic motor pattern is modified by a sensory feedback with

influences of central and peripheral afferents that control the chewing muscles during the most critical phases of mastication (Bosman et al., 2004).

The non-habitual mastication of Parafilm M is a standardized method, eliminating the interfering factors that act during the masticatory process, such as preferred side of mastication, swallowing between the chewing cycles, frequency of mastication and the texture of the food. In this study, in the clinical condition of non-habitual mastication with Parafilm M, there was a decrease of electromyographic activity in all muscles analysed for the hypertensive group. During non-habitual mastication of Parafilm M, moderate activity without the force is required from the muscles, so we can consider that the dentate individuals without disorders really are those with the more balanced musculature of the stomatognathic system (Siéssere et al., 2009).

The habitual, natural, non-oriented mastication pattern consists of alternating the side of work and is responsible for the fragmentation of food by the teeth by means of cyclic mandibular movements of opening and closing, called chewing cycles. Alterations caused by occlusion of the joints or muscles of mastication affect chewing efficiency (Kim et al., 1997). In mastication of soft (raisins) or hard (peanuts) foods, it was also found that the electromyographic values of the chewing cycles were lower for the hypertensive group.

The contraction of masticatory muscles is dynamic and successive during mastication. A critical component in regulating skeletal muscle contractility is the release of Ca^{2+} via ryanodine receptor (RyR) Ca^{2+} release channels in the sarcoplasmic reticulum (Andersson and Marks, 2010). There are indications that beta-blockers affect the skeletal muscle in therapeutic dosages (Finsterer and Gelpi, 2006).

Calcium channel blockers are commonly used in some cardiovascular disorders. These drugs can act at neuromuscular transmission, at both pre- and post-synaptic levels and may produce neuromuscular dysfunction. The calcium channel blockers, as verapamil and amlodipine, can impair neuromuscular transmission in individuals without neuromuscular disease, which may cause misinterpretation of single fiber electromyography studies carried out to investigate neuromuscular junction disorders (Ozkul, 2007; Seydi et al., 2020). In our study, 17% and 53% of hypertensive individuals make use calcium channel blockers and diuretics, respectively.

The efficacy and low cost of thiazide and thiazide-like diuretics become the most used as a potent medication option for many patients with hypertension (Duarte and Cooper-DeHoff, 2010). In our study, almost 100% of diuretic users has been treated with thiazide.

The members of the diuretic class of drugs vary greatly in structure, physicochemical properties and site and mechanism of action. Diuretics are drugs that increase the rate of urine flow and sodium (Na^+) excretion to adjust the volume and composition of body fluids (Cadwallader et al., 2010).

During excitation, muscle cells gain Na^+ and lose K^+, leading to a rise in extracellular K^+ ($[K^+]o$), depolarization, and loss of excitability. Recent studies support the idea that these events are important causes of muscle fatigue and that full use of the Na^+, K^+-ATPase (adenosine triphosphatase) (also known as the Na^+, K^+ pump) is often essential for adequate clearance of extracellular K^+ (Clausen, 2013).

In addition to the possible adverse effects of anti-hypertensive drugs in skeletal muscle, several changes in peripheral resistancy associated with hypertension may involve also the microvascular network (Vicaut, 2003). The supply of the tissues with oxygen, nutrients, and metabolites occurs almost exclusively in the microcirculation (Jung et al., 2013). In hypertension, the endothelial function and regulation of vascular tone is impaired with consequent increases in peripheral vascular resistance and inadequate regulation of oxygen supply to the skeletal muscle, which can affect muscle function (Nyberg et al., 2015).

The fact that the individuals with hypertension have lower electromyographic activity during mastication in comparison with the activity developed by the control individuals in the same clinical conditions, could indicate dysfunction of stomatognathic system caused by hypertension or by the anti-hypertension medication.

When muscle thickness was compared between the hypertensive and control groups, it was found that the thicknesses were quite similar for the masseter muscles, but for the temporal muscles, the values were quite different, being lower in the hypertensive group both at rest and at maximum voluntary contraction and these results are concurrent with international research (Bertram et al., 2003).

The morphological and functional characteristics of the muscles are different, where the masseter is a muscle potent with the function of force, which carries and maintains the bones, protecting and leading the power of the movement, and elevating the mandible anti-gravity during the various functions of the stomatognathic system. Temporal muscle function is more related to speed, being the first to contract when the mandible is closed, and considered to be a mandible positioner, adjusting the direction of movement, and acting as synchronizer of movements (Koolstra, 2002). The other researches show that muscles with a high proportion of fast fibers have a higher resistance than muscles rich in slow fibers and suggest that the type of fiber in skeletal muscle might be of importance for the development of the hypertensive disease (Juhlin-Dannfelt et al., 1979).

Although the muscle activity during chewing showed a decrease of electromyographic activity in all muscles analysed and the lower thickness for the temporal muscle for the hypertensive group, the similar bite force was verified between hypertensive and disease-free control group. The fact of bite force was not affected by the lower thickness of the temporal muscle in the hypertensive group individuals may be due to the ability of muscle fibers to adapt to new functional requirements to optimize their contractile function (Korfage et al., 2005).

In statistical analysis comparison between hypertensive and disease-free control groups, it was found that some factors such as body mass index, weight, age and height showed not significant differences within the group, only body mass index was a statistically significant factor among the groups.

It is known that the most hypertensive individuals are overweight, and one of the main recommendations for their treatment is to lose the excess weight. Further, clinical studies are necessary in order to define more precisely the risk factors for hypertensive diseases and the degree to which the stomatognathic system of hypertensive individuals is compromised, particularly assessing the type and duration of medication use, as well as time of hypertensive disease. Factors such as age, gender and body mass index should also be included in future longitudinal studies.

The present study was limited due to the group of patients with systemic arterial hypertension is very small, requiring further studies in this area of health.

Conclusion

The authors suggest that systemic arterial hypertension promotes functional changes of the masticatory system, especially with respect to its muscle activity during chewing and thickness for the temporal muscle, but without interference on the molar bite force.

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Fatal Neutropenic Colitis and *Clostridium Septicum* Bacteremia in a Breast Cancer Patient

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Abstract: A fatal case of 67-year-old female with metastatic breast cancer on chemotherapy complicated with febrile neutropenia, colitis and sepsis due to *Clostridium septicum* is presented. Important clinical symptoms, laboratory and radiology findings together with therapy and outcome of neuropenic colitis are also discussed.

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Introduction

Febrile neutropenia (FN) is serious complication of chemotherapy in cancer patients with solid tumours. As medical emergency FN must be swiftly managed including immediate administration of broad-spectrum bactericidal antibiotics with piperacillin/tazobactam being the most frequently used for the community-acquired and meropenem with vancomycin for hospital-acquired infections. Also, extensive work-up is an integral part of initial approach to a patient with FN. This consist of obtaining numerous blood cultures and specimens from different body sites as well as the use of imaging methods that may help to localize the focus of infection. The results of cultivation are obtained with a delay of one to two days; however, the detection of infectious focus with imaging methods is rapid having impact on choice of empirical antibiotic therapy, source control and assessment of the outcome.

Case report

A 67-year-old female presented to the emergency department three hours after acute onset of fever, chills, nausea, vomiting, lower abdominal pain, and bowel and

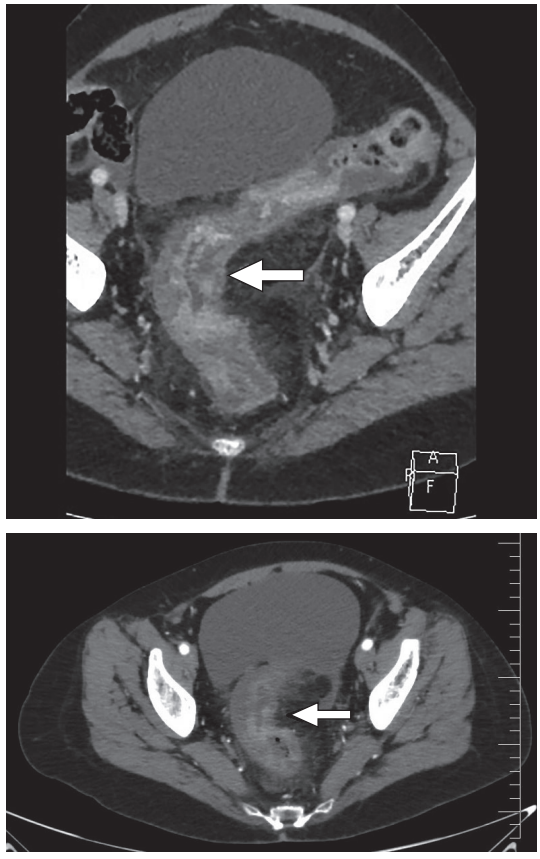

Figure 1 – a, b) Computed tomography scans of lower abdomen indicating sigmoid walls thickening.

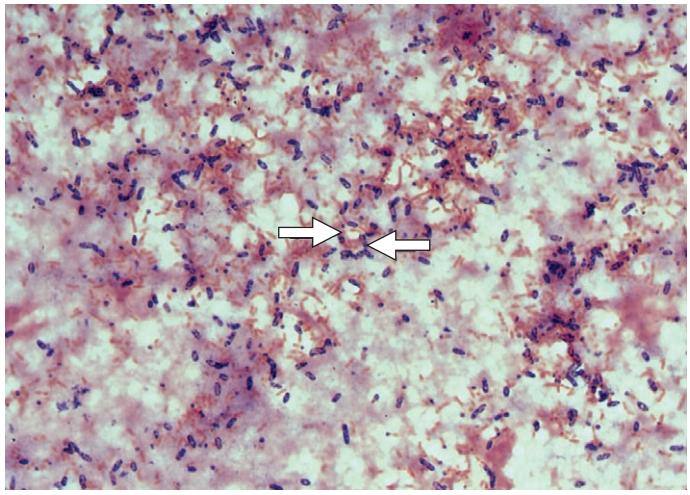

Figure 1 – c) Anaerobic blood culture bottle A with gas and colour of port wine and aerobic blood culture bottle B without bacterial growth; d) Gram stain of positive blood culture (arrows indicate Gram-positive rods).

urinary urgency. The patient had recently finished the first cycle of neoadjuvant chemotherapy with standard-dose epirubicin and cyclophosphamide for metastatic breast cancer. On examination, she was febrile with abdominal distension and suprapubic tenderness. Laboratory tests revealed a low hemoglobin level (108 g/l), leukopenia (0.71×10^9/l), neutropenia (0.13×10^9/l) and an elevated procalcitonin level (1.260 ng/ml). An underlying diagnosis of FN was established. Because of suspicion for acute abdomen, abdominal computed tomography was performed immediately with finding of colitis of the sigmoid colon to the rectum with suspicion of a developing abscess in the transition from the sigmoid colon to the rectum (Figure 1a and b). The patient received empirical antibiotic therapy with meropenem and was transferred to the intensive care unit where she died from septic shock two days later before surgery was possible. At that time, the anaerobic blood

culture demonstrated bacterial growth with gas production and hemolysis (Figure 1c) and Gram stain of the pre-cultivated blood from the blood culture bottle revealed numerous Gram-positive rods with a terminal spores and drumstick-like shape (Figure 1d). *Clostridium septicum* was identified from the blood culture and the diagnosis of clostridial bacteremia was established.

Discussion

Clostridium septicum is a highly virulent pathogen which is associated with colorectal malignancy, hematological malignancy, immunosuppression, diabetes mellitus and cyclical neutropenia. Clinical presentation of the infection may include sepsis, gas gangrene, and mycotic aortic aneurysms (Jessamy et al., 2016). Also, it is well known that clostridial bacteremia is associated with substantial mortality reaching 20% in cancer patients and *C. septicum* is the second most common cause of clostridial bacteremia in patients with solid tumours (Hammond et al., 2014). Nausea, vomiting, abdominal pain, hypotension, acute hemolysis and focal gastrointestinal signs are associated with increased 7-day mortality. Importantly, neutropenic colitis should always be considered in cancer patients receiving chemotherapy with FN and abdominal pain, because these complications represent a life-threatening condition requiring aggressive therapy including intravenous antibiotics and surgical intervention (Rodrigues et al., 2017). For antibiotic selection, usual anaerobic coverage can include penicillins, metronidazole or carbapenems. However, if clostridium has been identified, combination with clindamycin is preferred because it may block production of *C. septicum* toxin – the main virulent factor of the pathogen responsible for intravascular hemolysis and tissue necrosis (Stevens et al., 2014). It is worth noting that neutropenic colitis can be due to a variety of bacterial species with *Bacteroides* and *Clostridium* species being the most frequently isolated; however, when *C. septicum* is the cause of neutropenic colitis, sepsis and with a fulminant course and death commonly occur (Nesher and Rolston, 2013).

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Splenic Rupture and Massive Hemoperitoneum Due to Coagulopathy after *Atheris* Viper Snakebite

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Key words: Snakebite – *Atheris* viper – Coagulopathy – Splenic rupture – Hemoperitoneum – Splenectomy

Abstract: Coagulopathy with defibrination is one of symptoms accompanying snakebite envenoming, where life-threatening complications such as massive bleeding and organ hematomas formation can occur. Here, we report a case of hemocoagulation failure due to bite by African Great Lakes bush viper *Atheris nitschei* with impossibility of specific treatment for absence of antivenom and its life-threatening complication: very rare and unexpected atraumatic splenic rupture with massive hemoperitoneum and necessity of urgent splenectomy.

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Introduction

One of the snakebite envenoming manifestation is hemocoagulation failure: venom-induced consumption coagulopathy (VICC), most commonly with defibrination and subsequently possible bleeding and organ hematoma formations. This type of envenoming belongs to main venom's effects of snake's family *Viperidae* (not-European vipers, pit-vipers, rattlesnakes et al.). Besides of components affecting coagulation directly, in venom are present hemorrhagings and some other destructive enzymes that damage vascular endothelium and subendothelial tissue and are involved in the state of coagulation and the vascular system integrity during envenoming. The only effective final treatment for VICC is administration of appropriate antivenom.

In the case of African bush vipers *Atheris*, whose venom causes VICC, antivenom is not produced. Hence, in these cases, treatment is limited to mostly ineffective and not recommended fibrinogen (FBG) or fresh frozen plasma (FFP) substitution during several days of slow gradual spontaneous resolution of VICC, and eventual solution of emerging complications. Hemoperitoneum as a consequence of VICC is a relatively rare complication. Non-traumatic splenic rupture and bleeding after snakebite has been described so far in three cases, of which only in one case initially intact spleen with massive hemoperitoneum and splenectomy necessity, has occurred (Kang et al., 2014; Lal et al., 2014; Lee and Sung, 2019).

Case report

A 44-year-old man, an amateur snake breeder, was bitten into the 2nd finger of his right hand by the African Great Lakes bush viper *Atheris nitschei* (Figure 1). In about

Figure 1 – Great Lakes bush viper Atheris nitschei *(photo Jiroušek).*

an hour, he went to a specialized medical facility, the Toxinology Center of the General University Hospital in Prague, where he worked as a biochemist.

Affected hand was swollen, no clinical systemic symptoms were present. Anamnestically, he was observed for chronic glomerulonephritis.

In the first hemocoagulation examination 1 hour after the bite, only D-dimer 3,781 μg/l was recorded as pathological. Then, 5 hours after the bite, the laboratory finding already showed the development of VICC: INR (international normalized ratio) 3.8, APTT (activated partial thromboplastin time) 62.0 s, FBG < 0.1 g/l, D-dimer > 6,400 μg/l, finding of hemolysis. Next examination, 8 hours after the bite, already showed fully developed afibrinogenemia with INR > 10, APTT > 180 s, TT (thrombin time) > 180 s, D-dimer > 6,500 μg/l and hemolysis. Number of PLT (platelets) 214–216×10^9/l and antithrombin activities 65–70% did not deviate significantly from normal values, which corresponds with the common course of VICC. Leukocytosis (14–20×10^9/l) usually accompanies majority of snake envenoming.

As antivenom against *Atheris* viper venom is not produced, 6 TU (transfusion units) FFP were administered when FBG < 0.1 g/l. The effect was slight and transient, only in PT (prothrombin time) and TT (INR 3.8–2.5; TT > 180–63.4 s). Further examination showed again afibrinogenemia with immeasurable coagulation times. On the lower part of the right arm a hematoma formed. Due to the described positivity of the effect (Robinson et al., 2004) of formerly produced antivenom Near Middle East Antivenom Behringwerke (*Cerastes*, *Echis*, *Vipera* vipers), 3 vials of EchiTAb Clodomiro Picado (among others against *Echis* vipers) were administered as a test, but without any effect.

Laboratory findings of severe VICC with afibrinogenemia and unmeasurable coagulation persisted for 5 days, as well as hemolysis in some samples. At the same time, oliguric acute renal failure develops in grade III. according to KDIGO (Kidney Disease: Improving Global Outcomes) based on acute tubular necrosis in the field of already premorbid chronic glomerulonephritis with a creatinine peak of 395 μmol/l. During hospitalization, renal function gradually repaired without the need of renal replacement therapy, renal biopsy was not indicated.

From day 6, when coagulation values first appeared in the measurable range (FBG 0.14 g/l, INR 1.97, APTT 42.2 s, TT 27.1 s, D-dimer 5,024 μg/l), the patient was allowed to get out of bed for bathroom, contrary to previous strict sleep mode. Same day during defecation, weakness and vertigo suddenly developed. Diffuse palpable abdominal pain and hypotension (mean arterial pressure 55–60 mm Hg) were present, heart rate was stable, did not exceed 80/min. Urgent massive volume replacement therapy had not enough effective response; lower norepinephrine support (up to 0.12 μg/kg/min) was added. In laboratory findings Hb (hemoglobin) decline (97–74 g/l) and further progression of leukocytosis (11–16×10^9/l) after previous normalization were present. Bed side ultrasonography examination showed free fluid in the abdominal cavity, and immediate abdomen computer tomography

an obvious rupture of the spleen with massive hemoperitoneum. Norepinephrine support had to be increased temporarily up to 1 μg/kg/min.

Urgent revision of the abdominal cavity was indicated immediately, FBG 2 g, tranexamic acid 1 g were administered. Perioperatively found hemoperitoneum 3.5–4 l without blood clot formation and spleen rupture. Splenectomy performed. Another 2 g FBG, 6 TU RBC (red blood cells), 3 TU FFP, tranexamic acid 1 g were administered concurrently. During operation, the hemodynamic parameters gradually normalized, catecholamine support was reduced.

Spleen: 135×85×45 mm, in one edge with laceration of the capsule and parenchyma in the range of approximately 65×35×30 mm, in the adjacent approximately two thirds of the parenchyma dispersed multiple minor hemorrhages. Spleen capsule slightly dull. Microscopically, massive confluent fresh hemorrhages were found in the parenchyma. Rupture area without significant inflammatory reaction. Apart from hemorrhage, the structure of the parenchyma is usual with dominated congestion of the red pulp.

After the operation, the circulation parameters were normalized, catecholamine support was discontinued and Hb values corrected by transfusions to values of 90–100 g/l.

The further course was complicated on day 8 by bronchopneumonia, for which he was treated with antibiotics. Gradually occurring reactive thrombocytosis (308–1,230×10^9/l) as a result of splenectomy and infection was secured by acetylsalicylic acid 100 mg/day.

The patient was discharged in good health on day 17 after snakebite and admission.

Discussion

No specific antivenom is produced for the treatment of envenoming caused by *Atheris* vipers. The reasons are probably low incidence and mild course with low amount of bites complications in African territories of occurrence. The situation is similar in the case of snake's breeds. Literature description of envenoming is rare (Robinson et al., 2004; Top et al., 2006; Hatten et al., 2013). In 20-year-period, 5 cases of envenoming after *Atheris* bite were hospitalized and successfully treated without antivenom at the author's workplace (Valenta et al., 2014). Robinson et al. (2004) published a successful treatment using currently unproduced Antivenom Near Middle East Behrinwerke intended, among other, against the venom of the distantly related viper *Echis*. In our described case, however, a similar therapy with another antivenom against the African viper *Echis* proved ineffective.

FBG and FFP substitution with no antivenom treatment is not recommended. Present, not neutralized venom causes rapid destruction of FBG. The result is only an increase in fibrin degradation products, especially D-dimers. However, this procedure can be indicated in the case of the occurrence of serious bleeding, or its imminence during long-term afibrinogenemia (White, 2005; Berling and Isbister, 2015).

Renal injury accompanies a numbers of snakebite envenomations, especially with hemotoxic and myotoxic components in venom. Its origin is multifactorial, a participation has among others e.g. coagulation failure in VICC, hypotension, vascular infliction by hemorrhagins involvement, hemolysis with hemoglobinuria and others (Vikrant et al., 2017).

Although organ hematomas and spontaneous hemoperitoneum are not frequent complications of VICC, they may play a serious role in morbidity and lethality after snakebites in some localities of snake's occurrence (Berling and Isbister, 2015). Tchaou et al. (2016) reported in Benin (mostly saw-scaled vipers *Echis*) internal bleeding in 56% envenomed cases, in this number 22% of hematomas with hemoperitoneum and 12% hemoperitoneum cases. On other hand in Brazil Amazon, systemic bleeding was observed during hospitalization only in 15.3% lanceheads *Bothrops* snakebite patients with VICC, no hemoperitoneum included (Oliveira et al., 2019). Hemoperitoneum accompanying VICC is described rather sporadically (Rathod et al., 2003; Ahn et al., 2007; Diallo et al., 2019). Splenic rupture is then very rare complication (Kang et al., 2014; Lal et al., 2014).

Spleen tissue injury and its subsequent rupture in described case was contributed by more influences. Activated red pulp was probably congested, among others, even due to the number of hemolyzed erythrocytes. Also, the endothelial and subendothelial vascular injury caused by venoms hemorrhagines and other enzymes can be expected (Kang et al., 2014; Berling and Isbister, 2015). This, together with ongoing plasma coagulation system failure during VICC, caused numerous and confluent hemorrhages found in the parenchyma. Although the spleen has not been significantly enlarged, its more fragile structure could be expected. Probably, even with the participation of the abdominal pressure during defecation, there became a laceration of the parenchyma at the site of multiple hemorrhages, followed by rupture. Massive abdominal hemorrhage with incipient hemorrhagic shock requested urgent splenectomy.

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Oral Malignant Melanoma: A Case Report

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Key words: Malignant melanoma – Palate – Pigmentation – Melanocytes

Abstract: Melanoma is a malignant neoplasm of the epidermal melanocytes. Awareness and early recognition of pigmented lesion inside oral cavity helps in initial diagnosis and further investigation and treatment. Oral malignant melanoma is a rare aggressive neoplasm commonly seen among middle age. The diagnosis of melanoma initiates from the pre-existing pigmented lesions. The poor prognosis of oral melanomas requires that pigmented lesions of undetermined origin be routinely biopsied. A case of malignant melanoma of hard palate with its clinical, radiological and histopathological presentation along with brief review is presented. Prognosis of these lesion is poor with survival rate of 5 years.

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Introduction

Oral malignant melanomas are extremely rare lesions and occur commonly in the maxillary gingival and palatal region. Malignant melanoma is the third most common skin malignancy, yet it comprises of only 3 to 5% of all cutaneous malignancies (Boulaadas et al., 2007). Malignant melanomas of the oral cavity are extremely rare accounting for 0.2–8% of all malignant melanomas (Ebenezer, 2006; Guevara-Canales et al., 2012). Lesions are mainly very aggressive in nature but mostly go unnoticed as these lesions are clinically asymptomatic in the early stages and noted as hyperpigmented patch. Melanoma is a malignant tumour comprise of melanocytes in which cells are derived from the neural crest that constitute the melanin pigment in the basal and suprabasal layers of the epithelium (Ashok et al., 2020). Although most melanomas arise in the skin, they may also arise from mucosal surfaces (Deyhimi et al., 2017).

Case report

A 59-year-old male patient came to the department of oral medicine and radiology with the chief complaint of blackish pigmented area over the palate. Patient does not have symptom like pain or burning sensation, his main concern was aesthetic. Initially he noticed small patches of dark black pigmented area 6 months ago which increases to present size. There was a rapid spread of the hyperpigmented lesions which covered the entire palatal region. There was no familial history of carcinomas.

On clinical examination, the lesion was dark blackish to brownish in colour with irregular borders clearly demarcated from the adjacent area. Lesion covering left to centre part of hard palate extending from middle third to junction of hard and

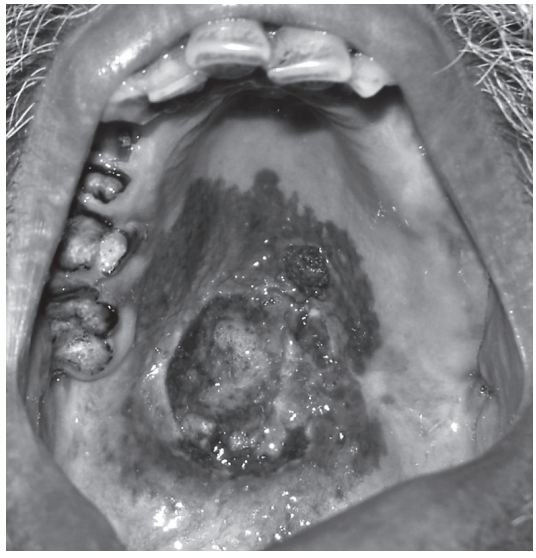

Figure 1 – A well-defined blackish brownish irregular shaped lesion with a nodular elevation in middle seen over hard palate.

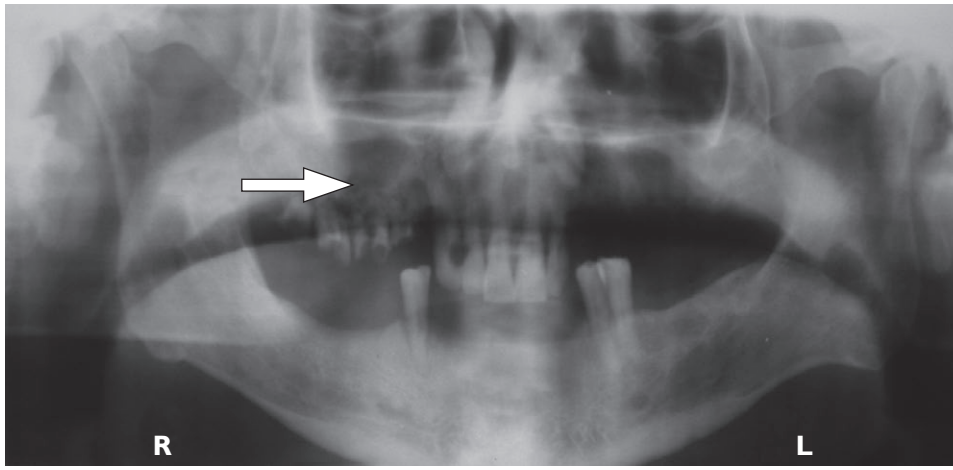

Figure 2 – Orthopantomograph showing a well-defined radiolucent oval right side of palate.

soft palate. Irregular in shape with ragged borders measuring around 3×3.5 cm. The surface appeared wrinkled, granular with a proliferative elevated area seen in midline palate (Figure 1). On palpation, the lesion was fibrotic non-scrapable and non-tender. The regional lymphnodes were not palpable.

Orthopantomograph (OPG) revels there is a well-defined radiolucency in right middle part of hard palate suggestive of bone erosion appearing as palatal loss (Figure 2). Under local anaesthesia a biopsy sample collected from proliferative region of hard palate.

In histopathological examination revels the given sift tissue H and E (haematoxylin and eosin) stained section shows parakeratininzed stratified squamous epithelium

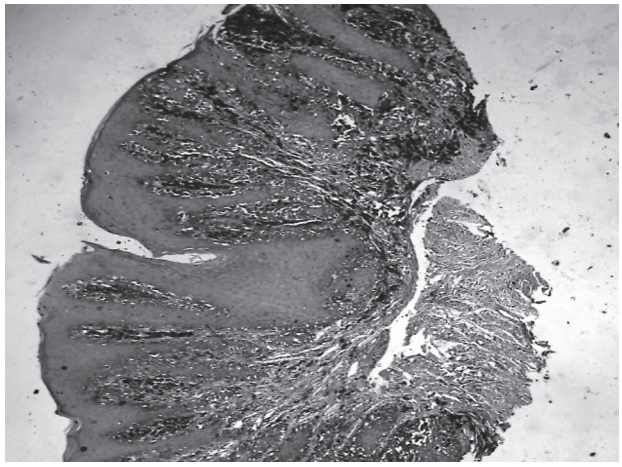

Figure 3 – Malignant melanocytes in epithelium and in stroma (under 4× microscope).

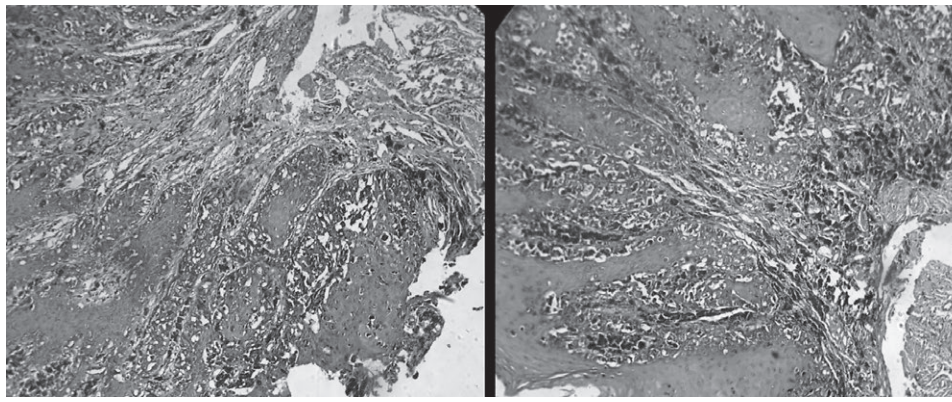

Figure 4 – Malignant melanocytes with dysplastic features in the epithelium as well as in stroma (under 10× microscope).

with dark brown coloured cells resembling melanocytes and are present throughout the epithelium and are arranged in nests and islands. Melanocytes are large and shows cellular pleomorphism, anisonucleosis and anisocytosis. Basal cell layer degeneration is seen with melanocytes invading into the connective tissue stroma. In the connective tissue, melanocytes are forming islands, and some are singly present. Stroma is dense with collagen fibres, fibroblasts and fibrocytes. Dilated blood vessels have also been reported with extravasated red blood cells. Focal areas of chronic inflammatory chiefly lymphocytes are also seen. Pigmentation is also noted in various places (Figures 3 and 4). Based on clinical, radiological and histopathological presentation its final diagnosed as malignant melanoma. Chest X-ray did not show any metastasis to lungs. A PET (position emission tomography) scan was performed, which did not show any distant metastasis. The patient was referred to oral and maxillofacial surgeon for excision of the lesion for further treatment and the prognosis of the lesion was explained. The patient wanted treatment in a different city and was referred for further management.

Discussion

Melanin is an endogenous non hematogenous pigment. It is initially formed by melanocytes in the basal layer of the epithelium and is transferred to adjacent keratinocytes via membrane-bound organelles called melanosomes. Benign lesions like common acquired nevus, congenital nevus, dysplastic nevus and cellular blue nevus are said to undergo a malignant transformation to melanoma. Mucosal melanoma was first described by Weber in 1895. Cigarette smoking, denture irritation and alcohol consumption are some of the suggested risk factors (Wu et al., 2016). Tobacco and formaldehyde exposures have also been suggested as causative agents for intraoral melanomas. Risk factors for melanoma include

Caucasian ancestry, fair skin, light hair and a history of intense sun exposure, and moles that are unusually numerous, large, irregular (Feller et al., 2017). Mostly it is seen between the ages of 40 to 70 years (Smith et al., 2016). Malignant melanoma is commonly seen in men compared to women (Singh et al., 2019). Most common site inside oral cavity is the palate followed by maxillary gingiva with an incidence of 80% and 91.4%, respectively (Lamichhane et al., 2015). It tends to rapidly spread to other parts of the body causing death.

Criteria for clinical diagnosis of melanoma (rule of ABCDE)

- Asymmetry – is when one-half of the lesion does not match the other half of lesion;
- Border irregularity – is when the edges are, notched, ragged or blurred;
- Colour irregularity – various coloured pigmentation is seen ranging from black, brown, tan, red, blue and white;
- Diameter – more than 6 mm;
- Elevation from the adjacent surface.

Melanoma can be categorised as different types as superficial spreading, nodular melanoma, lentigo maligna melanoma, acrallentiginous melanoma and mucosal lentiginous melanoma. Among these acrallentiginous and mucosal lentiginous melanoma commonly occur in the oral cavity. Hard palate is the most commonly involved site where it presents as a brown to black macule with irregular borders.

Surgery, chemotherapy, radiotherapy, and immunotherapy are preferred treatment. It has been shown that recent surgical excision followed by immuno-chemotherapy has reduced or prevented the recurrence of the lesion. The prognosis of oral melanoma is poor with a five-year survival of 15–38% of cases (Aloua et al., 2021). Cases where there is metastasis has already involved then the disease is considered as classically incurable, surgery, radiotherapy and chemotherapy could be considered under the palliative care.

Conclusion

A small, pigmented lesion inside oral cavity is very much misleading as it does not have any symptoms unless it became big and noted by the patient. Its early proper diagnosis can be life saving for the patient as it can be very aggressive and a invade to adjacent area.

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Testing Positive for SARS-CoV-2 in Two Countries 105 Days Apart

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Abstract: Recovered COVID-19 patients may test positive for SARS-CoV-2 for a long time from intermittent shedding of viral fragments. A 36-year-old man who tested positive for SARS-CoV-2 in the Czech Republic and recovered tested positive again in Bhutan, 105 days beyond his first positive test. He experienced minimal symptoms and recovered without complications. Although no virological test was conducted to rule out reinfection, the repeat positive test after initial recovery likely resulted from prolonged shedding of dead viral particles than a reinfection.

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Introduction

With a toll of 118,754,336 confirmed cases and 2,634,370 deaths globally as of 14th March 2021 (World Health Organization, 2021), the COVID-19 pandemic is either at its peak or in its second/third wave in different countries. As countries open up, concerns on an effective vaccine, lasting protective immunity and reinfection or reactivation have become critical. Initial suspicions of reinfection or reactivation from South Korea (Smith, 2020) and Switzerland (Ravioli et al., 2020) were refuted and explained by the detection of non-viable, non-infectious viral fragments for prolonged period in some recovered patients. However, reinfections have been established in Hongkong, Europe (Winter, 2020), India (Ghanekar, 2020) and the list is increasing with 64 confirmed cases as of 14th March 2021 (BNO News, 2020). However, the significance of reinfection in the purview of emerging mutations and vaccinations is debatable.

As part of its stringent COVID-19 preventive measures, Bhutan has instituted a 21-day mandatory facility quarantine of all people coming into the country since 31st March 2020. People in quarantine are tested on 3–5 days (by RT-PCR – Real-time Polymerase Chain Reaction), 13–14 days (by Rapid antibody test and RT-PCR) and on the completion of quarantine (by Rapid antibody test). Additionally, an individual is tested on arrival at the Point of Entry or any time during the quarantine (by RT-PCR), if symptomatic. Any individual confirmed to be positive for SARS-CoV-2 by a RT-PCR is isolated in government hospitals regardless of symptoms and severity. After isolation, patients are tested on day seven (asymptomatic) or after three days of symptom free period (symptomatic). Anyone with two negative RT-PCR reports 24 hours apart is deisolated into a government designated facility for another 14 days before discharging home.

We report a case of a 36-year-old Bhutanese man who tested positive for SARS-CoV-2 in the Czech Republic and recovered but tested positive again in Bhutan 105 days beyond the first positive test.

Case report

A 36-year-old Bhutanese man was in the Czech Republic on a student exchange program since the first week of March 2020. He shared accommodation in a two-room apartment with five students of different nationalities. Within a week after his arrival into the university, the Czech Republic went into a national lockdown due to the ongoing COVID-19 pandemic. By the end of March 2020, all of his five roommates tested positive for COVID-19 and isolated. He continued to stay in his residence and was tested positive on 3rd April 2020, by RT-PCR test. He was immediately isolated into a single room in the university's guest house. The individual claimed that he has never stepped out of his accommodation after the lockdown and suspected that he contracted the virus from his roommates.

At the time of testing positive, he did not have any symptoms except for a minimal dry cough which he had for some time even before his arrival into the Czech

Republic. However, after two days of isolation, he experienced loss of sense of smell, taste and loss of appetite which lasted for 3–4 days. He also had one episode of high fever on the third night of isolation which responded well to antipyretics. No other medications were provided. His cough became more frequent but was not worse. He thought drinking lots of green tea and hot water mixed with black pepper and ginger helped his throat and cough. After a week in isolation, he became symptom free and his taste, smell and appetite returned, although he still tested positive on 17th April 2020, two weeks after the first confirmation. Finally, after three weeks of isolation, he tested negative on 27th April 2020, and was de-isolated into a single occupancy in the university's dormitory until he departed for Bhutan on the 28th of June 2020. He arrived in Bhutan on the 29th of June 2020 and was directly put into facility quarantine, as per the existing protocol for arrivals into the country. During the quarantine, he did not have any signs and symptoms but underwent tests as per the quarantine testing protocol.

Test assays and results

At the time of testing the case, Bhutan was using the World Health Organization (WHO) approved primer sequences from Da An Gene Co., Ltd. (China) – Detection kit for 2019 Novel Coronavirus (2019-nCoV) – that targets ORF1ab and N genes of SARS-CoV-2. The kit includes an endogenous internal control detection system used for monitoring the processes of specimen collection, RNA and PCR amplification, and thereby reducing false negative results. The kit claimed an analytical sensitivity of 500 viral copies/ml. Any sample with an obvious amplification curve and a cycling threshold (Ct) value ≤ 40 was judged as positive for 2019 novel Coronavirus (Da An Gene Co., Ltd.).

Test assay details and report of tests conducted in the Czech Republic were not available. However, the individual confirmed that he was subjected to a nasopharyngeal swab sample for RT-PCR. He was not tested for SARS-CoV-2 antibody in the Czech Republic.

As per the existing testing protocol of facility quarantine, the individual was subjected to a RT-PCR on third day of quarantine which was negative. On the 14th day (July 16th, 2020), he underwent RT-PCR and Rapid antibody test (SD Biosensor, Korea). He tested positive for SARS-CoV-2 gene (Ct value of N gene was 31.9 and ORF1ab gene was 34.9) and for SARS-Covi-2 antibody (IgG), 105 days after his first positive test (3rd April to 16th July 2020). Following this, he was isolated into a hospital facility. He was deisolated on the 24th of July after testing twice negative in RT-PCR.

Discussion

To our knowledge, this was one of the first report of a COVID-19 patient testing positive again in 105 days after the first positive test, during the early period of the pandemic. In Bhutan's experience, laboratory confirmed COVID-19 cases took

about 12 days on average until they turn negative in RT-PCR (range 7–46 days), after the first confirmation. In one of the first reports of prolonged viral shedding, a 71-year-old woman in Wuhan was reported to have a documented viral shedding for up to 60 days from the onset of symptoms which was one of the longest durations of viral shedding reported (Li et al., 2020). There are several similar reports of prolonged viral shedding as well as testing positive again after initial negative test and recovery (Gousseff et al., 2020; Luo, 2020; Ravioli et al., 2020; Duggan et al., 2021). Concerns on whether these repeat positivity after initial clinical recovery or confirmed negative by RT-PCR were due to reinfections or reactivation have been refuted and explained as resulting from prolonged intermittent shedding of dead, non-viable virus in respiratory epithelial cell turn-over (Smith, 2020). Recently, there was growing concern on reinfection from mutated virus with reports from Hongkong, Europe and India (Ghanekar, 2020). Increasing number of evidences have confirmed the concept of reinfection in at least 64 cases to date (BNO News, 2020). However, confirming that the repeat positive case is due to reinfections with different strain of the virus require advanced laboratory capacity in culturing viruses from the cases and analysis through gene sequences which is not possible in many laboratories especially in developing countries such as Bhutan. Reinfections are scientifically confirmed only when genomic analysis of SARS-CoV-2 show genetically significant differences between each variant associated with each instance of infection. This was demonstrated in a 25-year-old male patient in Nevada (USA), who also experienced more severe symptoms compared to his first episode (Tillett et al., 2021).

Although reinfection was not ruled out, we opine that the case in description was a typical occurrence of prolonged and intermittent shedding of dead non-viable virus after clinical recovery. This is based on the high Ct value in RT-PCR, presence of IgG against SARS-CoV-2 and the individual remaining symptomless during confirmation. It is also due to such occurrence that many countries have shifted from test-based discharge to time-based discharge. The World Health Organization and countries such as Singapore and South Korea, recommend the discharge of COVID-19 patients between 14–21 days after the first laboratory confirmation (Ministry of Health, Singapore, 2020). Learning from these cases, Bhutan may need to adopt the time-based discharge criteria in order to avoid prolonged hospital stay of patients who may be shedding dead and non-infectious virus particles.

Testing of recovered patients are not recommended due to the occurrence of repeat positives after recovery and discharge leading to exhaustive public health control measures and diversion of diagnosis of other medical conditions, in countries like Singapore (Ministry of Health, Singapore, 2020). However, with recent evidence of reinfections, testing of recovered patients may need to be reviewed especially in a setting with wide community outbreak. Nonetheless, the fact that the case in description had no symptoms and was IgG positive with a definite history of testing negative after initial laboratory confirmation of infection should point largely towards

a prolonged shedding of dead viral fragments rather than reinfection of reactivation. Additionally, in the absence of laboratory capacity to perform viral load and gene sequencing, the Ct value (high in the described case) with seroconversion may be used to estimate the time or stage of infection and indications of recovery.

Conclusion

Despite increasing reports of reinfections in many countries, we conclude that the case in description was a typical case of prolonged shedding of viral particles after recovery from previous infection. However, with increasing reports of mutations and reinfections, counties should build clinical and virological expertise to exclude emergence of new variants and reinfection in the community.

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