Diplomarbeit

A prospective cohort study of cognitive function in patients with chronic hypoparathyroidism

eingereicht von

Mario Scherkl

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Universitätsklinik für Innere Medizin

Klinische Abteilung für Endokrinologie und Diabetologie

unter der Anleitung von

Dr.in med. univ. Adelina Tmava-Berisha
Priv.-Doz.in Dr.in med. univ. Karin Amrein, MSc.

Graz, am 08.09.2021

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 08.09.2021 *Mario Scherkl eh.*

Danksagungen

Beginnend möchte ich mich an dieser Stelle sehr herzlich bei meinen beiden Betreuerinnen Frau Dr.in med. univ. Adelina Tmava-Berisha und Frau Priv.-Doz.in Dr.in med. univ. Karin Amrein, MSc., für die ausgezeichnete Betreuung und Unterstützung bedanken, die weit über den Rahmen der eigentlichen Diplomarbeit hinausgingen. Sie standen mir stets mit fundierten Fachkenntnissen zur Seite und gaben mir die Möglichkeit, mich weitestgehend selbständig mit dem Thema zu befassen.

Explizit möchte ich mich hier bei Frau Priv.-Doz.in Dr.in med. univ. Karin Amrein, MSc. bedanken, die mir durch ihr Engagement und ihren Enthusiasmus für die Innere Medizin wesentliche Einblicke in Forschung und Wissenschaft gewährte. Neben Tätigkeiten an diversen Kongressen und Foren wurde mir die Gelegenheit geboten, mich aktiv am Schreiben von weiteren wissenschaftlichen Publikationen zu beteiligen.

Ein besonderer Dank gilt meiner Frau Lisa, die mich durch die Höhen und Tiefen der vergangenen Jahre begleitete. Erst durch ihren Rückhalt und ihre Geduld konnte ich die Tiefen bewältigen und die Höhen feiern.

Außerdem möchte ich sowohl meiner als auch Lisas Familie danken, die mich allesamt vom oft stressigen Studiendasein ablenkten und mir die Ausgewogenheit zukommen ließen, die meines Erachtens notwendig für das erfolgreiche Studium der Humanmedizin war.

Table of Contents

Glossary and Abbreviations

AIRE	Autoimmune regulator
APS-1	Autoimmune polyendocrine syndrome type 1
BGC	Basal ganglia calcification
CaSR	Calcium-sensing receptor
CT	Computer tomography
HLA	Human leukocyte antigen
IQ	Intelligence quotient
MMSE	Mini Mental State Examination
MMT-B	Munich Memory Test B
MRI	Magnetic resonance imaging
MWT-B	Multiple-Choice Vocabulary Test B
PTH	Parathyroid hormone
QoL	Quality of Life
RANK	Receptor Activator of NF-κB
RANKL	Receptor Activator of NF-κB Ligand
rhPTH	Recombinant human parathyroid hormone
SF-12	Short-Form 12-Item Survey
SF-MPQ	Short-Form McGill Pain Questionnaire
VDR	Vitamin D receptor

List of Figures

List of Tables

Zusammenfassung

Hintergrund

Chronischer Hypoparathyreoidismus ist eine seltene endokrine Erkrankung, die meist postoperativ nach chirurgischen Eingriffen im Halsbereich auftritt. Beeinträchtigtes Nebenschilddrüsengewebe bedingt einen Ausfall der physiologischen Parathormonsekretion mit konsekutiver Hypokalziämie und Hyperphosphatämie. Ergänzend zu den klassischen Symptomen wie Tetanien und Parästhesien können mögliche Spätmanifestationen trotz leitlinienkonformer Standardtherapie weitreichend sein, ein Zusammenhang zwischen Hypoparathyreoidismus und kognitiven Dysfunktionen wie Gedächtnisstörungen und Konzentrationsschwierigkeiten wird diskutiert. Ziel dieser Arbeit ist es, Aussagen über die Prävalenz von kognitiven Dysfunktionen bei betroffenen Personen treffen zu können.

Methoden

Im Zeitraum vom Dezember 2019 bis Jänner 2021 wurden 10 volljährige Patientinnen und Patienten mit chronischem Hypoparathyreoidismus in unsere prospektive Beobachtungsstudie eingeschlossen. Die primäre Auswahl der Testpersonen erfolgte durch die Klinische Abteilung für Endokrinologie und Diabetologie des LKH-Univ. Klinikums Graz.

In Einzelgesprächen wurden neurokognitive Screening-Verfahren wie der Mini-Mental-Status-Test (MMSE), der Multiple-Choice-Vokabeltest B (MWT-B) und der Münchner Gedächtnistest B (MMT-B) angewandt, um die Auswirkungen von Hypoparathyreoidismus auf die Gedächtnisleistung zu prüfen.

Zur Auswertung der Testungen wurden die Ergebnisse der Studiengruppe mit den Testergebnissen einer nicht exponierten Kontrollgruppe verglichen.

Resultate

Bei 9 von 10 an der Studie beteiligten Testpersonen handelte es sich um Frauen. Das mittlere Alter bei Krankheitsbeginn betrug 34,9 (± 12,4) Jahre, und die neurokognitive

Testung wurde nach einer mittleren Krankheitsdauer von 8,2 (± 8,5) Jahren durchgeführt.

Der globale MMSE-Score der Studiengruppe von 29,5 (± 0,8) Punkten war ähnlich hoch dem MMSE-Score der Kontrollgruppe mit 29,7 (± 0,6) Punkten. Keine Testperson hatte einen Score unter dem Schwellenwert von 24 Punkten, welcher auf eingeschränkte kognitive Funktion hinweisen würde. Laut dem MWT-B lag der mittlere geschätzte Intelligenzquotient (IQ) der Studiengruppe bei 106 (± 10,2) und damit über dem mittleren Rohwert der Normierungsstichprobe von 100.

Die statistische Auswertung der Testergebnisse zeigte keine Korrelation zwischen chronischem Hypoparathyreoidismus und kognitiver Dysfunktion.

Schlussfolgerungen

Kognitive Dysfunktion bei langjährigem chronischen Hypoparathyreoidismus kann laut aktueller Literatur eine durch die Grunderkrankung ohnehin beeinträchtige Lebensqualität weiter einschränken.

Allerdings stehen noch zu wenige Daten über die pathophysiologischen Zusammenhänge dieser beiden Krankheitsentitäten zur Verfügung. In unserer kleinen Stichprobe zeigte sich kein deutlicher Unterschied zwischen Hypoparathyreoidismus-Betroffenen und einer Kontrollgruppe. Weitere Studien sollten neben der Befundung von Laborwerten und bildgebenden CT- und MRI-Aufnahmen zusätzlich neurokognitive Testungen durchführen, um auf mögliche Zusammenhänge zwischen PTH-Mangel und kognitiver Dysfunktion zu schließen. Außerdem sollten die Vor- und Nachteile einer therapeutischen PTH-Substitution genauer evaluiert werden.

Abstract

Background

Chronic hypoparathyroidism is a rare endocrine disorder, characterized by inadequately low parathyroid hormone (PTH) levels in the blood. It typically occurs postoperatively after impairment of parathyroid tissue in the setting of neck surgeries.

Possible late manifestations of consecutive calcium and phosphate disturbances are severe and may lead to intracranial calcification and cognitive dysfunction. Affected patients report frequent concentration deficits ranging from mild cognitive impairment to dementia. The aim of this thesis was to determine the prevalence of cognitive dysfunction in adult patients with chronic hypoparathyroidism.

Methods

In this prospective cohort study, a battery of neurocognitive tests including the Mini Mental State Examination (MMSE), the Multiple-Choice Vocabulary Test B (MWT-B), and the Munich Memory Test B (MMT-B), was used to assess general cognitive functions.

In the period from December 2019 to January 2021, 10 adult patients with diagnosed hypoparathyroidism could be identified as study participants and underwent neurocognitive assessment. The results were compared with the test results of a non-exposed patient group.

Results

9 out of 10 study participants were women. The mean age at onset of the disease was 34.9 (± 12.4) years, and the neurocognitive assessment was performed after an average of 8.2 (± 8.5) years of disease duration.

The global MMSE score of the study group of 29.5 (± 0.8) was similar to the control group with 29.7 (± 0.6) points. None of the study participants had a score below the threshold of 24, which would indicate impaired cognitive function. According to the

MWT-B, the mean estimated intelligence quotient (IQ) of the study group was 106 (± 10.2) and therefore above the raw score of the norming sample, defined as 100.

The statistical evaluation of the test results showed no correlation between chronic hypoparathyroidism and cognitive dysfunction.

Conclusions

According to the current literature, chronic hypoparathyroidism may be associated with cognitive dysfunction. However, there is currently only limited data available on the pathophysiological link between these two entities. In our small sample size, no substantial difference was seen between patients and controls.

Further studies including functional CT and MRI testing need to provide laboratory and neuroimaging evidence along with cognitive assessment to support an association between PTH deficiency and impaired cognitive functions. Also, the therapeutic effects of PTH replacement should be addressed.

1 Introduction

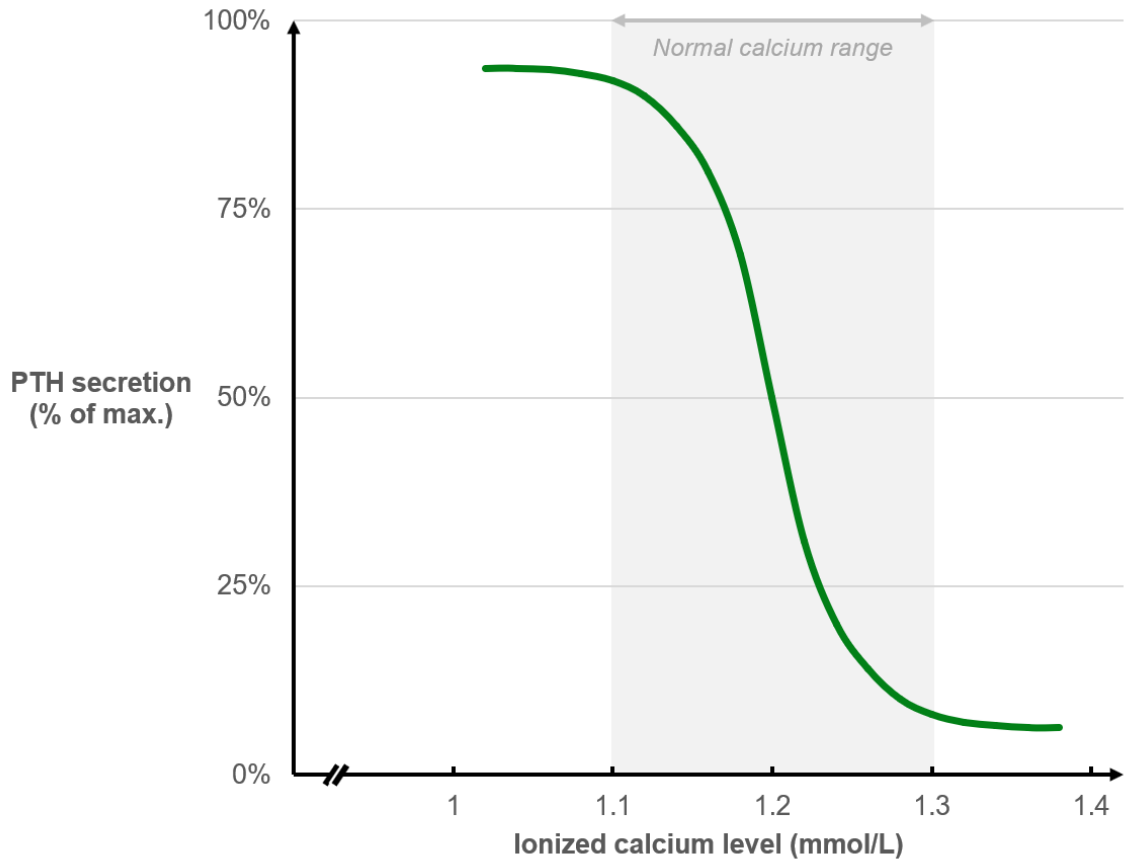
1.1 Parathyroid Hormone (PTH)

The parathyroid hormone (PTH) is an endocrine hormone produced by the main cells of the parathyroid glands, which are located dorsal to the thyroid gland in the lower neck area. The active form of PTH consists of a single polypeptide chain (84 amino acids) and results after the cleavage from pre-pro-PTH (115 amino acids) to pro-PTH (90 amino acids) [1]. The biological in-vivo half-life of PTH is short. After 3 to 5 minutes, the polypeptide chain breaks down into fragments and gets cleared mainly by the liver, the kidneys, and partly by the parathyroid glands themselves [2].

1.1.1 Regulation of PTH

The main stimulus for the secretion of PTH is a decrease in ionized calcium level in the blood, which is mainly mediated by calcium-sensing receptors (CaSR) of the epithelial cells of the parathyroid glands [3,4]. After being released into the bloodstream, PTH binds to G-protein-coupled receptors on the cell surface of bone, kidney, and nerve tissue within a few seconds and promotes a range of physiological processes to maintain calcium and phosphate homeostasis [2]. In contrast to increased PTH secretion in hypocalcemic states, high levels of ionized calcium inhibit the release of PTH through a negative feedback mechanism. The amount of intact PTH available, which normally ranges between 10 to 65 ng/L [2], is therefore highly dependent on the ionized calcium level in the blood. The inverse sigmoidal relationship of these two components is shown in **Figure 1**.

Figure 1: The inverse sigmoidal relationship between PTH level and ionized calcium level in the blood. Low ionized calcium levels increase PTH secretion and vice versa.



1.1.2 Effects of PTH

PTH is crucial for maintaining optimal serum calcium and serum phosphate levels by acting on multiple organ systems.

In bone tissue, PTH binds to PTH receptors of osteoblasts, resulting in enhanced production of RANKL (Receptor Activator of NF-κB Ligand) and decreased secretion of osteoprotegerin. RANKL then binds to the transmembrane receptor RANK (Receptor Activator of NF-κB) on the surface of immature osteoclasts, induces cell differentiation and increases osteoclastic activity. The interaction of osteoblasts and osteoclasts is essential for the ongoing process of bone remodeling and bone health. Normally, osteoprotegerin would inhibit osteoclastic cell differentiation by preventing the binding from RANKL to RANK. This inhibition fails in PTH-related osteoprotegerin deficiency. The increased osteoclastic activity induces osteolysis, leading to the upregulated dissolution of hard mineralized hydroxyapatite in the bones and thereby to the release of calcium and phosphate. As soon as the positively charged calcium

ions and the negatively charged phosphate ions are released from the bones, they bind together again in the blood, meaning that the relative proportion of free ionized calcium in the total serum calcium does not increase at the beginning.

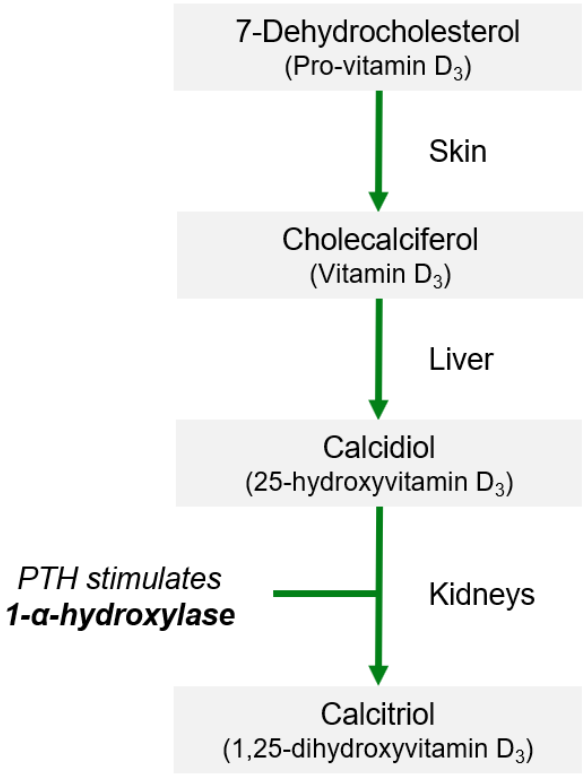
In proximal tubular cells of the kidney, PTH inhibits the reabsorption of phosphate by downregulating the sodium-phosphate-transporters. This causes more phosphate to get excreted renally, increasing the relative amount of free ionized calcium in the blood [5]. At the same time, PTH increases calcium reabsorption in the Henle loops of the kidney through the incorporation of calcium channels and stimulation of sodium-calcium channels [3], in a way that the free ionized calcium mobilized from the bones remains in the blood. PTH, therefore, acts anticalciuretic and inhibits the excretion of calcium into the urine.

1.1.3 Role of Calcitriol (Vitamin D_3)

In the long term, a PTH-induced dissolution of hydroxyapatite leads to progressive demineralization of the bones, followed by severe osteolytic complications like rickets, osteomalacia, and osteoporosis [6].

To prevent bone loss, the body enhances intestinal absorption of dietary calcium and phosphate. This mechanism is mainly regulated by the steroid hormone calcitriol. Calcitriol (or 1,25-dihydroxyvitamin D_3) is the biologically most active form of vitamin D. It is formed in the kidneys after hydroxylation of calcidiol (or 25-hydroxyvitamin D_3) by the action of an enzyme called 1-α-hydroxylase [3,7]. PTH stimulates renal 1-α-hydroxylase, whose activity represents the final biochemical step in the calcitriol biosynthesis, as shown in **Figure 2**. Additionally, low calcium and phosphate levels also stimulate 1-α-hydroxylase, resulting in upregulated calcitriol secretion.

Figure 2: PTH stimulates renal 1-α-hydroxylase and enables calcitriol biosynthesis.



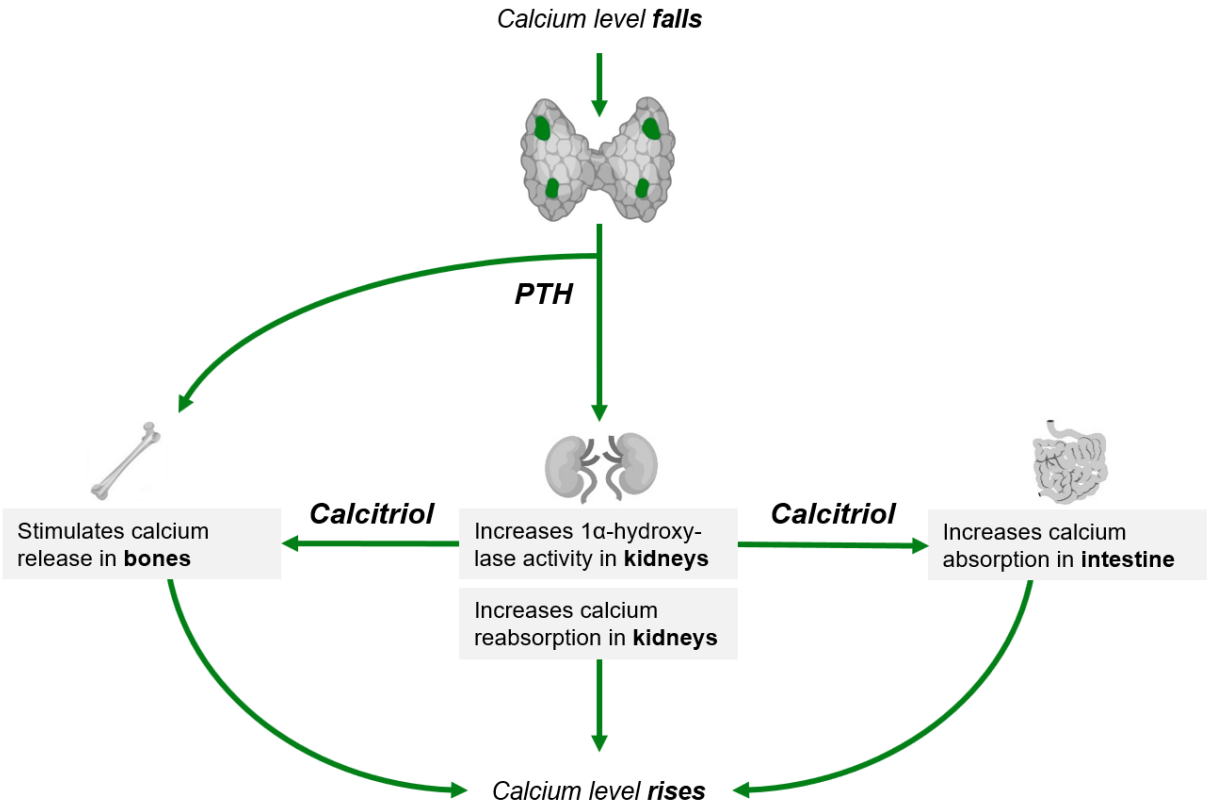
Calcitriol acts on three major mechanisms: intestinal absorption, bone metabolism, and renal reabsorption.

Calcitriol is the main mediator for active and thus energy-dependent transcellular calcium transport from the intestinal lumen into the blood [8]. After binding to intracellular vitamin D receptors (VDR) of intestinal epithelial cells, calcitriol promotes transcriptional activation of calcium-binding protein genes [9,10]. Higher levels of this calcium-binding protein cause the opening of calcium channels and increase the active transport of calcium across the intestinal mucosa. Under physiological conditions, 70 to 80 % of dietary calcium is absorbed in the ileal segment of the small intestine [11]. Calcitriol is also involved in increasing the active intestinal absorption of phosphate through sodium-dependent phosphate co-transporters [12,13]. This enables the body to absorb most of the necessary phosphate through dietary intake.

In the kidneys, calcitriol also binds to VDR and promotes glomerular filtration and tubular reabsorption of calcium. Conversely, an excess of calcium ions in the blood can be compensated by increased renal excretion [14]. It is discussed whether calcitriol increases renal phosphate absorption in the presence of PTH [15]. A better explanation would be the simultaneous effect of PTH to inhibit renal phosphate reabsorption.

Calcitriol has direct and indirect effects on the activity of bone cells. Indirectly, calcitriol promotes the incorporation of hydroxyapatite into the bone matrix via increased calcium and phosphate supply after intestinal absorption. Like PTH, calcitriol also interacts with osteoblasts and increases the membrane expression of RANKL, which in turn converts the preosteoclast to a mature osteoclast [16]. Elevated osteoblastic and osteoclastic activity leads to elevated bone metabolism and supports the maintenance of optimal calcium and phosphate levels in the blood. The correlations of PTH and calcitriol were graphically illustrated in **Figure 3**. PTH increases the production and release of calcitriol from the kidney, whereas a lack of calcitriol upregulates the production and release of PTH in the parathyroid glands.

Figure 3: The most important hormonal control elements of the calcium balance are PTH and the steroid hormone calcitriol.



1.1.4 Role of Calcitonin

Another key player in calcium homeostasis is the hypocalcemic peptide hormone calcitonin, which is mainly secreted by parafollicular C-cells of the thyroid gland [17]. Calcitonin is a functional antagonist of PTH and lowers the calcium level in the blood. So, unlike PTH, the secretion of calcitonin is stimulated by calcium levels above the normal range. The normal range for total serum calcium is 2.2 to 2.6 mmol/L. The main effects of calcitonin are enhanced renal calcium excretion and the direct inhibition of osteoclast-mediated bone resorption in response to the binding of calcitonin to G-protein-coupled receptors of osteoclasts [17]. Due to these bone-protective properties, forms of calcitonin are used therapeutically to prevent excessive bone loss. It has proven itself in the treatment of hypercalcemia, osteoporosis, and bone metastases [18]. A tightly controlled feedback loop between the endocrine hormones PTH, calcitriol, and calcitonin and its effect on intestine, kidney, and bone is of great importance for calcium and phosphate homeostasis.

1.2 Epidemiology

Defined in the clinical guidelines of the European Society of Endocrinology, chronic hypoparathyroidism is a rare endocrine disorder that lasts for more than 6 months and is characterized by parathyroid insufficiency and inappropriately low PTH level in the blood [19]. Due to its rarity, chronic hypoparathyroidism is listed as an orphan disease. The incidence and prevalence of the disease in the general population is not entirely clear, as it affects individuals of any age.

1.2.1 Etiology

The most common cause of chronic hypoparathyroidism is due to surgical removal, trauma, or devascularization of parathyroid tissue following thyroidectomy, parathyroidectomy, or other operations in the anterior neck area. Depending on the literature, between 68 % and 75 % of all hypoparathyroid cases are affected by the postoperative form [20-22]. Prevalent indications for surgical interventions are thyroid adenoma and carcinoma, thyroid goiter, and Graves' disease. Total thyroidectomy was once considered the treatment of choice for neoplastic thyroid diseases [23]. This radical

therapy option is nowadays strongly debated. Paradoxically, it can be said that the surgical treatment of primary hyperparathyroidism could lead to postoperative hypoparathyroidism if too much parathyroid tissue gets damaged. Impaired parathyroid tissue is no longer capable of producing adequate amounts of intact PTH. The deficiency can be either transient or permanent. The latter is called chronic hypoparathyroidism if it persists for more than 6 months after surgery and, according to a review from Sinnott in 2018, only affects about 3 % of patients [24]. However, only very few patients become symptomatic, as in most patients the secretion of PTH in the parathyroid glands recovers after a while. In 79 % of transient cases, parathyroid function recovers in the first month after surgery. Significant predictor for the recovery of parathyroid function was the number of in situ remaining parathyroid glands, as some patients showed PTH synthesis again after one year of postoperative hypoparathyroidism [25]. Early prevention of hypocalcemia by rapid calcium substitution is associated with better outcome [26].

Less common causes of chronic hypoparathyroidism are due to autoimmune or genetic disorders. A mutation in the autoimmune regulator (AIRE) gene causes a misleading immune response of autoantibodies against endocrine tissue. To be more specific, it is assumed that autoantibodies activate extracellular CaSR, resulting in reduced PTH secretion and immunomodulated destruction of parathyroid tissue [22,24]. Autoimmune hypoparathyroidism may occur either isolated or as part of autoimmune polyendocrine syndrome type 1 (APS-1) [20]. The isolated occurrence only affects the parathyroid glands, whereas APS-1 further affects endocrine organs like the thyroid gland, the kidneys, and the gonads.

Another genetic cause can be found in DiGeorge syndrome. A microdeletion in chromosome 22 leads to a hypoplastic thymus, congenital heart diseases, and maldeveloped parathyroid glands [27]. The mutation in DiGeorge syndrome is one of the most common chromosomal defects, estimated to occur in 1 in 4,000 newborns [28-30]. In 60 % of cases, persistent hypoparathyroidism develops [31]. Maldeveloped parathyroid glands cannot fulfill the body's need for PTH, resulting in hypoparathyroid states with hypocalcemia and hyperphosphatemia. Due to the hypoplastic thymus, patients show T-cell deficiency and increased susceptibility to infections.

Both severe magnesium deficiency and excess may limit the release of PTH and its effect on the end organs. Moderate decreased magnesium levels in the blood

stimulates PTH secretion, as magnesium can exert similar effects as calcium to receptors of the epithelial cells of the parathyroid glands. But severe magnesium deficiency can induce paradoxical inhibition of PTH release due to misactivated CaSR activity [32]. In contrary, high serum magnesium levels increase thyroid calcitonin secretion to stimulate renal magnesium excretion. As a functional antagonist of PTH, calcitonin inhibits PTH secretion. This reversible form of hypoparathyroidism can be corrected by maintaining adequate magnesium levels [22].

Rare, acquired causes are radioactive iodine therapy for thyroid diseases or extensive radiation therapy as part of a cancer treatment [27]. Also, cancer itself could infiltrate the parathyroid glands and impair their function.

The symptoms of parathyroid insufficiency can occur without a lack of PTH. This is known as pseudohypoparathyroidism and is due to a genetically determined resistance of the body to PTH in which the end organs do not respond adequately to the PTH present [22]. In contrast to all other forms of hypoparathyroidism, elevated PTH levels can usually be detected in the blood despite hypocalcemia and hyperphosphatemia.

If the underlying cause for the present hypoparathyroidism cannot be found, the term idiopathic hypoparathyroidism is used [20].

1.2.2 Affected Population

The few epidemiologic studies available to date report an estimated prevalence of 22 to 37 per 100,000 person-years in western countries [27,33]. Approximately 70 to 90 % of patients affected by chronic hypoparathyroidism are female [27,33,34]. Women are also affected more often by autoimmune thyroid diseases and therefore undergo neck surgery more frequently. The complex interaction between thyroid hormones and female sex hormones is suspected as a possible cause for the frequent occurrence of thyroid diseases in women. According to a multicenter observational study, the mean age at diagnosis of hypoparathyroidism is 45.5 years, with a mean duration of disease of 9 years [34]. These results are in line with the statements of other studies reporting that 75 % of patients are older than 45 years [35,36]. With increasing age and duration of the disease, pathological deposition of calcium-phosphate products leading to basal ganglia calcification and nephrocalcinosis, as well as limitations in glomerular filtration rate are observed more frequently [37].

In most cases, previous neck surgery can be identified as the primary cause of hypoparathyroidism. Postoperative hypoparathyroidism may occur shortly after neck surgery or appear after a latent interval of months to years [22]. A retrospective analysis of 105 postoperative hypoparathyroidism patients from the University Hospital Graz showed, that the disease was diagnosed in mean 5.5 years after the first surgical intervention [38]. In non-surgical hypoparathyroidism, the disease can be diagnosed much earlier at a median age of 17 months [39]. The pediatric forms of hypoparathyroidism are mostly due to genetic and congenital disorders, affecting males and females in equal numbers.

1.3 Consequences of PTH Deficiency

Long-lasting PTH deficiency massively affects calcium and phosphate homeostasis and causes lower calcium and increased phosphate levels in the blood, leading to the clinical manifestations of hypoparathyroidism with neurological, cognitive, muscular, and cardiac complications. The intensity of complications correlates with the severity of the metabolic disturbance but can also remain asymptomatic for years in cases of slow development. In contrast, rapid onset of severe hypocalcemic symptoms in postoperative settings must be treated quickly.

1.3.1 Effects on Calcium Metabolism

Calcium is the most abundant mineral in the human body. About 99 % of the body's calcium is bound to phosphate and stored in bones and teeth in the form of hydroxyapatite, a mineral with the formula $Ca_5(PO_4)_3(OH)$ [40]. Hydroxyapatite is responsible for the strength and stability of bones and acts as a mineral storage for calcium and phosphate. The remaining 1 % of the calcium in the body can be found in the intra- and extracellular space and is tightly regulated by the endocrine hormones PTH, calcitriol, and calcitonin circulating in the bloodstream. Besides its important role in bone formation, calcium is also involved in a large number of vital tasks, like initiation of vascular contraction and dilatation, nerve signal conduction, neuromuscular excitability, blood clotting, and apoptosis [41].

According to international consensus, a normal level of total serum calcium should be in a range between 2.1 to 2.6 mmol/L in the presence of normal plasma protein concentrations [41,42]. Approximately 45 % of it, which corresponds to 1.1 to 1.3 mmol/L, is available in the form of ionized calcium. Ionized calcium, also known as free calcium, is the most active form of calcium and the metabolic important fraction of total serum calcium that is not attached to proteins or other minerals. The remaining serum calcium is either bound to proteins like albumin, the main carrier of protein-bound calcium, or forms complexes with small molecules like phosphate. As a result, the total serum calcium also varies with the level of albumin and may need to be corrected mathematically. A decrease of albumin by 1 g/dl causes a decrease in total serum calcium by about 1 mg/dl. The relative ratio of bound calcium to free ionized calcium also depends on the acid-base balance. In alkaline conditions, it increases in favor of the bound calcium, as more free calcium ions bind to negatively charged albumin.

Despite its necessity for the human body, calcium cannot be produced by the organism itself and must be supplied from outside via the diet. According to the Austrian Society for Nutrition, a daily intake of 1000 mg of calcium seems to be sufficient for young adults, pregnant women, and the elderly [43]. If the dietary intake cannot provide sufficient calcium or calcium absorption through the intestine is impaired, the endocrine hormones PTH and calcitriol are used to dissolve stored calcium from the bones. Impaired intestinal calcium absorption may be the result of vitamin D deficiency as described in **Chapter 1.3.3**.

1.3.1.1 Hypocalcemia

Hypocalcemia is defined as a total serum calcium level less than 2.1 mmol/L. In the context of chronic hypoparathyroidism, a decreased calcium release from the bones induced by PTH deficiency is not the primary cause of hypocalcemia. The general lack of anticalciuretic PTH and thereby inhibited 1-α-hydroxylase activity in the kidney leading to reduced calcitriol biosynthesis is more severe. Consequently, less calcitriol binds to intracellular VDR of intestinal epithelial cells, which in turn prevents adequate calcium absorption across the intestinal mucosa.

In cases of acutely falling calcium levels, neuromuscular and cardiac complications may occur. The symptoms highly correlate with the severity of hypocalcemia. Despite

similar laboratory values, not all patients show the same clinical manifestation to the same extent.

1.3.1.2 Neuromuscular Hyperexcitability

Compared to the extracellular space, the intracellular calcium level is between 0.0001 to 0.0002 mmol/L and therefore 10,000 times lower [44]. The different calcium levels cause a difference in charge across the cell membrane. Necessary for the triggering of action potentials in nerve, skeletal muscle, and heart muscle cells is the crossing of stimulus thresholds. A stimulus threshold is the smallest voltage that triggers an action potential. During an action potential, voltage-dependent sodium channels are opened, and the following influx of sodium ions causes a charge shift in the cell. This so-called depolarization is necessary for the axonal transmission of neuronal stimuli to other excitable cells [45]. By activating CaSR signaling, extracellular calcium increases the stimulus threshold of sodium channels and consequently blocks the influx of sodium ions and axonal transmission. Low serum calcium levels enhance neuromuscular excitability by reducing this blockage and allowing spontaneous discharges in nerves. This neuromuscular hyperexcitability leads to a nervous affliction called tetany.

1.3.1.3 Signs and Symptoms

The manifestations of hypocalcemic tetany may range from mild symptoms such as plain restlessness, fatigue, perioral numbness, or paresthesias in fingers and toes to more painful muscle cramps and seizures [42]. Common clinical signs of neuromuscular hyperexcitability, which may show up on physical examination, are the Chvostek's sign and the Trousseau's sign [46,47]. The Chvostek's sign, named after the Austrian doctor Franz Chvostek, is tested by tapping along the course of the facial nerve. If the facial muscles contract or twitch, the Chvostek's sign is positive. Whereas the Trousseau's sign is characterized by carpal spasm triggered by applying a blood pressure cuff for a maximum of 3 minutes, which leads to a typical obstetrician's hand with painful flexion of the wrist and extended interphalangeal joints in tetanic conditions. In literature, the Trousseau's sign is attributed a higher sensitivity and specificity than Chvostek's sign [48].

Calcium plays a key role in myocardial contractility. Profound hypocalcemia can lead to bradycardia and measurable prolongation of the QTc interval in the electrocardiogram (ECG), predisposing to ventricular arrhythmias and low cardiac output in severe cases. Hypocalcemia is therefore a rare but reversible cause of heart failure, even without an underlying myocardial disease [49]. The threshold for physiological QTc intervals varies between 440 and 500 milliseconds, depending on the literature, gender, and heartbeat [50-52]. A prolonged QTc interval in the ECG can be an incidental finding during routine examinations and may provide the first clinical sign of hypocalcemia.

1.3.2 Hyperphosphatemia

Hyperphosphatemia is defined as an elevated serum phosphate level greater than 1.46 mmol/L [53,54]. The regulation of phosphate, which is involved in intracellular energy production and formation of bone and teeth, is closely linked to the regulation of calcium, and depends on the endocrine hormones PTH, calcitriol, and calcitonin. The consideration of related regulatory mechanisms of calcium and phosphate is important since approximately 99 % of the body's calcium is bound to phosphate in the form of hydroxyapatite.

In the context of hypoparathyroidism and PTH deficiency, the inhibition of renal phosphate reabsorption is downregulated, leading to an elevated phosphate level in the blood. However, symptoms directly related to high serum phosphate levels are rare, as the clinical presentation of chronic hypoparathyroidism is usually caused by the concomitant hypocalcemia. Jono et al. reported already in 2000, that in in vitro models, a serum phosphate level above 2 mmol/L can dramatically increase calcium precipitation in smooth muscle cells [55]. As soon as the solubility product of positively charged calcium ions and negatively charged phosphate ions is exceeded due to high serum phosphate levels, it may come to an increased deposition of extraskeletal calcium-phosphate products in soft and connective tissue, blood vessels, and joints. In addition, the risk of stone and calculus formation in the kidneys increases. Severe consequences of vascular calcifications are stenosis of the blood vessels and cardiac, central, and peripheral circulatory disorders. Although the exact mechanisms are still not clear, it is assumed that ectopic deposition of calcium-phosphate products in the

basal ganglia and other areas of the brain is responsible for the occurrence of cognitive dysfunctions [56]. The deposition of calcium-phosphate products causes the relative proportion of free ionized calcium in the blood to fall, which further intensifies preexisting hypocalcemia.

1.3.3 Vitamin D Deficiency

A 25-hydroxyvitamin D_3 (abbreviated 25(OH)D_3) level in the blood below 50 nmol/L or 20 ng/ml is referred to as vitamin D deficiency [57]. There is increasing scientific data associating vitamin D deficiency with cognitive dysfunction and vascular dementia [58]. Besides its crucial role in calcium metabolism, an adequate vitamin D status seems to have a protective effect against chronic illness and neurocognitive and mental disorders, as VDR are also expressed on immune cells [59]. Vitamin D also plays an important role in the terminal differentiation of promyelocytes into monocytes, which in turn differentiate into macrophages as part of the immune system [60]. Vitamin D deficiency is linked to inducing autoimmune diseases, increases susceptibility to infections, and predicts poor clinical outcomes in critical illness [61].

In conditions of PTH deficiency, a lack of PTH cannot sustain the final step in the vitamin D biosynthesis due to the missing stimulation of the 1-α-hydroxylase. As a result, a decreased vitamin D level cannot meet the physiological needs of vitamin D. Vitamin D deficiency results in bone health complications like rickets in children and osteomalacia in adults. The clinical signs of the diseases are bone pain, muscle weakness, and deformed long bones that bow under body weight. A long-term effect of vitamin D and calcium deficiency is a condition named osteoporosis, a skeletal disorder characterized by reduced bone mineral density and an increased risk for bone fractures. Under physiological conditions, a lack of vitamin D would enhance PTH secretion. The increased amounts of circulating PTH explains the excessive calcium release from the bones and the bone-weakening consequences [62]. However, since PTH secretion is impaired in hypoparathyroidism, bone loss is attributed to the lack of intestinal calcium uptake and inhibited bone incorporation of calcium due to vitamin D deficiency. At the same time, reduced renal reabsorption of calcium leads to hypercalciuria and an increased risk of urinary stone and calculus formation. All these

factors cause a drop in serum calcium level which cannot be compensated for due to the failing PTH secretion.

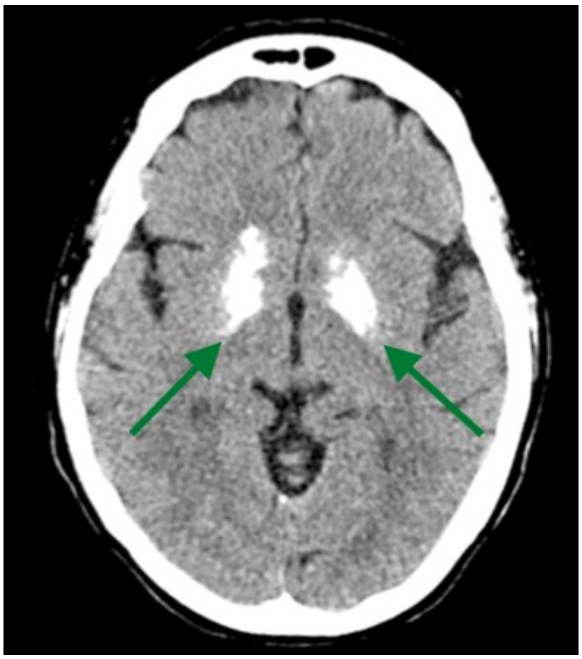
1.4 Neurocognitive Dysfunction

The common clinical features of hypoparathyroidism like paresthesia, tetany, and seizures are mainly due to hypocalcemia. Longstanding metabolic disturbances in chronic hypoparathyroidism are associated with severe complications of the central nervous system, including depression, dementia, cognitive dysfunction, and impaired executive functions. It is estimated that at least half of patients with postoperative hypoparathyroidism experience psychiatric symptoms during the disease [63]. Mental disorders like hallucinations, states of confusion, and psychoses are rare.

1.4.1 Basal Ganglia Calcification

The basal ganglia are a cluster of subcortical nuclei in the brain. As part of the extrapyramidal system, they play a key role in the regulation of motor, cognitive, and executive functions [64]. Basal ganglia calcification (BGC), the ectopic deposition of calcium-phosphate products due to a longstanding hyperphosphatemia and abnormal serum calcium levels, can damage the walls of arterioles, veins, and capillaries in affected brain regions. Damaged blood vessels of the basal ganglia may explain the cognitive dysfunction linked to chronic hypoparathyroidism. The most common clinical manifestations are impaired memory performance, dementia, seizures, and deterioration of extrapyramidal motor functions and speech [56,65]. However, the mentioned symptoms do not correlate exclusively with the extent of BGC. Rather, it may be the combination of intracranial calcification and hypocalcemia that exacerbates the clinical presentation, as Aggarwal et al. revealed total serum calcium and calcium-phosphate products to be independent predictors for cognitive dysfunctions [56,66]. It is believed that hyperphosphatemia activates inorganic phosphate transporters and leads to an increased expression of osteogenic molecules in the grey matter of the brain, resulting in BGC [67]. Due to the improvement in neuroimaging in the last years, these intracranial calcifications can be revealed more frequently, as a CT scan in **Figure 4** shows [65].

Figure 4: Symmetric basal ganglia calcification visualized in a CT scan of the head. The calcified areas appear brightened. Image from: Donzuso et al., 2019



Chronic hypoparathyroidism resulting in a disrupted calcium-phosphate ratio appears to be one of the most common causes of BGC [46,68,69]. However, the pathogenic significance of abnormal serum calcium levels is not fully understood, as both hypocalcemia (as a consequence of hypoparathyroidism) and hypercalcemia (after overtreatment of hypoparathyroidism with calcium and overdosed vitamin D_3) are also linked to neurocognitive impairment and may mask underlying BGC [65,67]. The need to focus primarily on the high serum phosphate levels when correcting a disturbed calcium-phosphate ratio is discussed.

To a certain extent, physiological calcifications seem to occur more frequently with increasing age. They are usually clinically silent and were detected in 20 % of all routine CT scans of asymptomatic patients [68]. The prevalence of BGC appears to be higher in patients with hypoparathyroidism, as studies reported more than a half (52 % by Shoback et al. 2016, 73.8 % by Goswami et al. 2012) of hypoparathyroid cases showing BGC on CT and MRI scans [67,70]. The clinical finding of BGC revealed in CT and MRI scans combined with progressive cognitive dysfunction indicates a neurodegenerative clinical picture named Fahr's disease.

1.4.2 Epileptic Seizures

Epilepsy is the name of a functional disorder of the central nervous system characterized by abnormal electrical activity in the brain, which is not triggered by a currently existing, identifiable cause. A seizure is an isolated clinical event that can occur as part of epilepsy. Paroxysmal episodes of depolarization provoke a sudden rush of electrical activity in neurons and other excitable cells, causing unusual behavior, sensations, and loss of awareness. Current literature and multiple case reports record that up to 60 % of patients with chronic hypoparathyroidism have experienced episodes of epileptic seizures [70-72]. Hypocalcemic triggered seizures may be the first clinical sign of the endocrine disease [73]. But it is still unknown whether and to what extent metabolic disturbances are responsible for the seizures. Intracranial calcification can be neglected as the main cause of epilepsy, as BGC was detectable in only 0.6 % of all epilepsy patients [74]. Another argument against BGC as a cause of epilepsy is that seizures can improve despite the progression of calcium-phosphate deposits [70]. Due to the similar clinical features of epilepsy and hypoparathyroidism, there is a risk that hypocalcemia-related seizures may be wrongly assigned to epilepsy, and vice versa. Seedat et al. reported of a young man who was treated for epilepsy for over 10 years until further laboratory tests and intracranial calcifications in neuroimaging identified idiopathic hypoparathyroidism as the actual and treatable cause of the recurrent seizures [75].

1.4.3 Dementia

An increased prevalence of dementia occurs in up to 65 % of patients with hypoparathyroidism compared to a normal control group [76]. Dementia is an overall term for progressive and abnormal changes of the brain characterized by the loss of memory. Starting with mild cognitive impairment, patients suffering from hypoparathyroidism and dementia later complain of frequent memory gaps, situations of disorientation, and speech disorders like aphasia [77]. These devastating features make it difficult to complete daily routines and have a profound impact on patients and their families on a social, emotional, and financial level. The associations between hypoparathyroidism and dementia are not yet clear, as vascular dementia is more common in patients with high serum PTH levels [77]. Although PTH is capable of crossing

the blood-brain barrier and PTH receptors are found in the human brain [78], it is assumed that the dementia conditions are mostly due to the calcium imbalance and not to PTH itself [35].

1.4.4 Brain Fog

Brain fog is a made-up word that is used to describe the subjective inability or difficulty of affected patients to concentrate and pay attention. The term is commonly used by patients with hypoparathyroidism to describe cognitive impairment [22]. In contrast to dementia, memory is less affected. However, brain fog might be an early sign of a process that leads to dementia. Kowdley et al. revealed cognitive impairment in 7 out of 11 hypoparathyroid patients [76]. In case-control studies, patients with chronic hypoparathyroidism showed significantly higher cognitive impairment than controls (32,3 % vs 5,7 %) [56]. Impairments were mainly memory recall, decision-making, and attention processes. It is also reported that cognitive dysfunction increased with both longer duration of the disease and increased presence of calcium-phosphate products in the blood. The severity and form of impairment also depend on the calcified areas of the brain [79].

1.4.5 Psychiatric Disease

Underbjerg et al. noted a significantly higher incidence of neuropsychiatric complications in terms of depression or bipolar affective disorders in patients with hypoparathyroidism, along with an increased risk of more frequent and longer hospitalization [80]. The incidence of depression and other types of psychiatric diseases was twice as high in patients with postoperative hypoparathyroidism (3.1 %) as in a non-exposed control group (1.5 %). Higher scores for depression were also found in patients with postoperative and nonsurgical hypoparathyroidism by other studies [81,82].

Hoogendijk et al. assessed the connection between PTH, vitamin D, and the occurrence of depression and concluded that depression is more associated with decreased vitamin D levels and high circulating PTH levels in the blood [83]. Consequently, in hypoparathyroidism, vitamin D deficiency should be assigned a higher significance in the development of depression. Compromised QoL due to chronic hypoparathyroidism may exacerbate preexisting depressive episodes [84]. A

case of a female patient who suffered from depression for years reports, that the depression disappeared after restoring calcium balance using calcium supplements [85]. But it is still unclear whether the effects are directly or indirectly related to calcium metabolism or to other, yet unexplored mechanisms of vitamin D.

1.5 Treatment

Chronic hypoparathyroidism can be a permanent condition that requires lifelong treatment. The treatment depends upon the clinical features and severity of serum calcium disturbances. Acute and chronic hypocalcemia must be treated differently, as the severity of symptoms also depends on the speed of development of the hypocalcemia [24].

The current standard therapy consists of supplementing calcium and vitamin D_3 rather than replacing the missing PTH, as synthetic recombinant human PTH (rhPTH) is not recommended as 1st line therapy. This is also since rhPTH (1-84) has only been used in the treatment of chronic hypoparathyroidism for a few years and long-term evidence is still missing. While the American Food and Drug Administration approval already took place in 2015, rhPTH was only approved by the European Medicines Agency in 2017 [24,86]. Synthetic rhPTH (1-84) is currently commercially available under the name Natpar® in Germany, Greece, Austria, Denmark, and Norway [87]. Dietary intake of calcium and phosphate should be optimized according to the guidelines. Usually, serum phosphate levels normalize with the administration of calcium and vitamin D_3, so no special diets are needed. To avoid secondary diseases such as hypercalcemia or kidney failure, regular check-ups are required. Biochemical monitoring includes ionized and albumin-corrected calcium, phosphate, and magnesium in the blood. Urinary calcium excretion and serum creatinine levels are assessed to evaluate renal function.

1.5.1 Conventional Treatment

The current standard treatment of chronic hypoparathyroidism includes the use of calcium supplements and active and native vitamin D_3 metabolites to achieve albumin-corrected serum calcium levels in the lower normal range [35]. The complexity of the

treatment requires individualized therapies, which are primarily based on the patients' symptoms and well-being, and less on the laboratory values. In addition to the standard treatment, magnesium and phosphate supplements, as well as thiazide diuretics are used as needed [88].

In acutely symptomatic patients, intravenous treatment using 1-2 g calcium gluconate in 50 ml 5% glucose solution must be given over 10-20 minutes to prevent life-threatening epileptic seizures or cardiac arrhythmias followed by a calcium gluconate drip of 1 mg/ml with a rate of 50 mg/hour [89]. Even though both the doses and the duration of administration vary in the literature, it is always started with a higher initial dose and then reduced. To prevent the precipitation of calcium-phosphate products in the blood, phosphate and bicarbonate must not be added. A switch from intravenous to oral supplementation of calcium and vitamin D_3 should be initiated as soon as possible [24].

Chronic hypocalcemia without acute clinical features can be treated with oral administration of 1.5-2 g calcium (carbonate and/or citrate) [89]. Calcium supplements can be divided into multiple doses per day to allow better intestinal absorption. The European Society of Endocrinology also recommends the use of the vitamin D_3 metabolites cholecalciferol (vitamin D_3), calcitriol (1,25-dihydroxyvitamin D_3), and alfacalcidiol (1-hydroxycholecalciferol) [86]. The active vitamin D_3 metabolite calcitriol offers better controllability of serum calcium levels than native cholecalciferol due to its shorter half-life of 4-6 hours. Simultaneous administration of native and active vitamin D_3 is preferred. Due to PTH deficiency in hypoparathyroidism, the lack of stimulation of renal calcium reabsorption should be noted, leading to increased calcium excretion and a higher risk of nephrocalcinosis and chronic kidney disease. To avoid hypercalciuria, serum calcium and phosphate levels must be monitored regularly. Annual measurement of 24-h urinary calcium excretion should also be considered [86]. In cases of persistent hypercalciuria, calcium-sparing thiazide diuretics may be useful to reduce calcium supplementation.

1.5.2 Hormone Replacement Therapy

In patients who do not achieve serum calcium in the lower normal range and improvement of the symptoms, hormone replacement therapy using rhPTH should be

considered. However, the primary therapy goal is not normocalcemia, but a reduction in the necessary calcium and vitamin D_3 supplementation. Both the truncated rhPTH (1-34) and the longer intact rhPTH (1-84) are synthetic analogs of the endogenous PTH. They are supposed to be injected subcutaneously into the thigh or abdomen by patients once per day with a starting dose of 50 µg. The daily dose of rhPTH infusion can be adjusted between 25 and 100 µg in individual cases [86].

1.5.2.1 Teriparatide [rhPTH (1-34)]

rhPTH (1-34) is primarily approved for osteoporosis management. Unlike common medication used to treat osteoporosis, rhPTH (1-34) acts osteoanabolic and promotes bone formation by binding to PTH receptors on the cell surface of osteoblasts. This increases bone mineral density in the presence of sufficient calcium and vitamin D and reduces the risk of osteoporotic fractures. Recent evidence showed that normal serum calcium levels can be maintained in response to rhPTH (1-34) replacement therapy. Besides, the use of synthetic PTH is also useful to prevent side effects from excessive calcium and vitamin D_3 administration [90]. In randomized crossover trials from Winer et al., a significant reduction of 59 % in urine calcium excretion was observed after rhPTH (1-34) was continuously administered using infusion pumps, compared to injections twice a day [91]. The twice-daily injections of rhPTH (1-34) showed less fluctuation in serum calcium levels compared to the once-daily injection [92].

1.5.2.2 Natpar [rhPTH (1-84)]

rhPTH (1-84), the full-length molecular form of PTH consisting of 84 amino acids, is indicated as an adjunct to calcium and vitamin D_3 in symptomatic patients with chronic hypoparathyroidism and albumin-corrected serum calcium level < 2.0 mmol/L. It is used when standard therapy does not show satisfactory results. Studies confirm the reduced need for supplemental calcium and vitamin D_3 and the increase in bone mineral density under rhPTH (1-84) administration [24,93].

A replacement of a missing hormone seems obvious, but the guidelines recommend against routine use of rhPTH (1-84) [86]. Due to the short half-life of endogenous PTH and the unphysiological once-daily injection of its artificial form, the PTH levels can not

be regulated sustainably. In addition, there is a lack of physiological adaptation to external and internal factors such as pregnancy, varying physical activity, new-onset diseases, or shifts in calcium balance. Regarding the once-daily injection, a double-blind, placebo-controlled, randomized, phase 3 study reported that the proportion of patients who had adverse events such as hypocalcemia, muscle cramps, or paresthesia was the same in rhPTH (1-84) and placebo groups (11 % vs 9 % of patients) [94]. The primary endpoint of the study, defined as at least 50% reduction in initial daily calcium and vitamin D_3 supplementation plus serum calcium levels above initial baseline levels, was much higher in the rhPTH (1-84) group compared to the placebo group (53 % vs 2 % of patients). Improved well-being under rhPTH (1-84) use has been reported in several case reports [86].

1.5.3 Surgical Intervention

A surgical approach to manage hypoparathyroidism is the transplantation of parathyroid tissue. It can be a definitive treatment option and avoids the side effects of long-term medication with calcium supplements and vitamin D_3 metabolites.

Autologous transplantation allows intact parathyroid tissue to be transplanted into separate muscle pockets of the brachioradialis or sternocleidomastoid muscle to preserve function [95,96]. It can be used as a preventive measure when parathyroid tissue may be damaged during neck surgery or to protect against damage from postoperative radiation therapy. Preservation of intact parathyroid tissue during surgical procedures is desirable but not always possible. Another alternative to treat chronic hypoparathyroidism is parathyroid allotransplantation. Living-donor excision and transplantation into patients showed promising results despite short-term need for immunosuppressive therapy [97]. Available evidence suggests elevated PTH and calcium levels in the blood 24 months after surgery [98].

2 Aims and Objectives

2.1 Hypothesis

1. There seems to be an association between chronic hypoparathyroidism and cognitive dysfunction, which further impairs executive functions and semantic verbal learning and memory. The resulting socio-economic effects, such as unemployment or social withdrawal, can further reduce the QoL of patients.

2. A health-related reduced QoL reported by patients is often underestimated both by medical staff and non-exposed individuals, leading to the perception of an empathy gap.

2.2 Aim

The aim of this thesis was to determine the prevalence of cognitive dysfunction in adult patients with diagnosed chronic hypoparathyroidism. The focus was on testing general neurocognitive deficits, estimating premorbid crystallized intelligence, and clarification semantic verbal learning and memory.

Literature was reviewed and compared to the own results to provide information on the diagnosis, clinical presentations, and complications. Furthermore, statements of this thesis are intended to raise awareness of the reduced QoL in chronic hypoparathyroidism.

3 Materials and Methods

3.1 Study Character

This prospective cohort study was approved by the Institutional Ethics Committee of the Medical University of Graz, vote 29-062 ex 16/17. A battery of neurocognitive tests was used to assess general cognitive functions, verbal learning memory, and crystalline intelligence. A study group with patients suffering from chronic hypoparathyroidism and a control group with non-exposed individuals were formed. After explaining the use of this study and signing the consent form, all participants were subjected to neurocognitive testing in individual interviews. All parts of the tests were conducted on paper, while the statistical analysis of the results was done digitally using Microsoft Excel and the analysis software IBM SPSS.

To evaluate the tests, the results of the study group were compared with the test results of the control group to draw possible conclusions about the impact of chronic hypoparathyroidism on neurocognitive dysfunction.

3.2 Patient Collective

3.2.1 Study Group

In the period from December 2019 to January 2021, 10 patients (9 women and 1 man) with diagnosed hypoparathyroidism could be identified as study participants. The primary filtering was carried out by the Division of Endocrinology and Diabetology of the University Hospital Graz. The age of the patients ranged from 24 to 58 years, giving a mean age of 43.1 (± 13.2) years for the study group. All patients gave written consent to take part in this observational study. After signing the consent form, the patients were included as participants and assigned to the study group.

3.2.2 Control Group

The control group consists of 10 non-exposed individuals, mean age 47.10 (± 8.0) years, without hypoparathyroidism who matched the study group in terms of age and gender. All participants in the control group gave their informed consent for

participation in the study and were mainly recruited from the private or clinic-related environment.

3.2.3 Limitation

The results of this thesis are primarily limited by the small sample size and the purely observational study character. Another limiting factor is that no CT or MRI findings of the brain were collected. The presence and extent of possible BGC could therefore not be determined. No physical examination was performed that could indicate extrapyramidal motor dysfunctions.

Furthermore, the level of education from the patients was not comparable between study and control groups due to a lack of information. It must also be pointed out that not all neurocognitive test parts performed in the interviews were evaluated statistically. In this thesis, the **Mini Mental State Examination** (MMSE), the **Multiple-Choice Vocabulary Intelligence Test B** (MWT-B), and the **Munich Memory Test B** (MMT-B) were preferred.

Only patients over 18 years of age were included in this prospective cohort study. To carry out a valid verbal test series, the ability to read and understand the German language was required. Hypoparathyroidism had to be present for at least six months to document a chronic state, and already known mental disorders or cognitive impairment had to be excluded in advance. Data collection for the HYPAUS study [34] continued after the statistical analysis of 20 study participants for this thesis.

3.2.4 Collected Patient Data

At the beginning of the neurocognitive assessment, handwritten general information and currently prescribed medication from the participants of the study group were collected. The participants also had to fill out questionnaires about their current QoL (SF-12) and pain (SF-MPQ), their social and professional environment, and their symptoms while suffering from hypoparathyroidism. The information collected is described in more detail in **Table 1** and **Table 2**.

Table 1: General information collected from the participants.

Item		Question type
Name		Short answer
Date of birth		Short answer
Profession		Short answer
Workload		Short answer
Height		Short answer
Weight		Short answer
Comorbidities		Short answer
Medication		Short answer
Marital status		Multiple choice (single, in a partnership, married, divorced, widowed)
Women only	Births	Short answer
	Pregnancies	Short answer

Table 2: Collected information related to chronic hypoparathyroidism.

Item	Question type
Date of diagnosis of hypoparathyroidism	Short answer
Reduction of workload since the onset of disease	Multiple choice (yes, no)
Age at onset of disease	Short answer
Etiology	Multiple choice (postoperative, autoimmune, after radioiodine therapy, genetic, idiopathic)
Quality of Life (SF-12) and Pain (SF-MPQ)	Rating scale
Therapy	Multiple responses, Short answer
Symptoms of calcium deficiency	Multiple responses, Short answer
Acute complications due to hypocalcemia	Multiple responses, Short answer

Cardiovascular symptoms	Multiple responses, Short answer
Neuropsychiatric manifestations	Multiple responses, Short answer
Visual performance	Multiple responses, Short answer
Kidneys and urinary calcium	Multiple responses, Short answer
Hospitalization	Multiple responses, Short answer
Infections	Multiple responses, Short answer
Fractures	Multiple responses, Short answer
Bone density	Multiple responses, Short answer
Cranial imaging	Multiple responses, Short answer
Dental status	Multiple responses, Short answer
Other subjectively or objectively relevant problems	Short essay

3.3 Neurocognitive assessment

All participants underwent neurocognitive assessment in individual interviews. The interviews lasted about 1 hour on average and were conducted in a confidential atmosphere. The neurocognitive test battery included the **Mini Mental State Examination** (MMSE), the **Multiple-Choice Vocabulary Intelligence Test B** (MWT-B), and the **Munich Memory Test B** (MMT-B). All test parts were always applied in German translation and in a fixed order, taking required waiting times between test parts into account. For this reason, the MMT-B had to be divided into several parts. The timetable of the test parts can be found in **Table 3**.

3.3.1 Mini Mental State Examination (MMSE)

The MMSE is the most used brief cognitive screening test for initial detection, assessment, and follow-up of cognitive deficits in clinical and research settings [99]. Using a simple paper-based questionnaire, functions such as orientation, attention, memory, and the ability to follow simple commands are assessed by points and added up to a total score. The participants take a passive role and only respond to the

questions and tasks read out by the interviewer. The score scale ranges from 0 to 30 points, whereby a maximum score of 30 points represents unrestricted cognitive functions. The threshold for normal cognitive functions is usually set to a score of 24 points. However, the cut point for the threshold varies in several studies [100]. Lower scores indicating more severe cognitive deficits and are seen in the presence of at least mild dementia.

The MMSE is not suitable for diagnosing the form of dementia and cannot make any statement about the cause of the cognitive deficits. Besides neurodegenerative brain changes associated with dementia, depression can also lead to considerable limitations in cognitive functions, which are reflected in lowered test scores. The MMSE usually takes about 5 to 10 minutes.

3.3.2 Multiple-Choice Vocabulary Test B (MWT-B)

The MWT-B is a verbal performance test for estimating premorbid crystallized intelligence and correlates to a high degree with the verbal intelligence quotient (IQ) of a tested person [101]. This enables the MWT-B to collect an objective sample of knowledge that is hardly influenced by mental disorders.

37 rows of 5 German words each are presented, whereby the correct written word must be identified. The level of difficulty increases from word series to word series. The total number of correctly identified words is compared with the performance of a representative German-speaking control group to derive the estimated IQ.

3.3.3 Munich Memory Test B (MMT-B)

The MMT-B is the German adaptation of the California Verbal Learning Test and is used for the general clarification of semantic verbal learning and memory. By memorizing and recalling word lists, the semantic short and long-term memory is observed, which can subsequently lead to conclusions about neurocognitive impairments.

A learning list (“Monday list”) with 16 words, which can be classified in 4 categories of 4 words each, is read out by the examiner in a fixed order over five learning trials. After each trial, the study participant is asked to recall as many words as possible. This is

followed by the one-time reading and recalling of an interference list ("Tuesday list"), which also consists of 4 categories of 4 words each. Two categories from the interference list have already appeared in the learning list. It follows a **short-delayed free recall** and a **short-delayed cued recall** of the learning list. In cued recall, the examiner will mention the 4 categories beforehand. After about 20 minutes the memorized words are queried again in a **long-delayed free recall** and a **long-delayed cued recall** of the learning list. Finally, the test ends with a **long-delayed recognition** task. The examiner reads out 44 words, and the test person is asked to identify the words that were also present in the learning list.

Table 3: Chronological order of the neurocognitive tests performed in an interview.

Test	Description
MMSE	Initial detection of cognitive deficits.
MWT-B	Measurement of premorbid IQ.
MMT-B (Part 1) Short delay free recall Short delay cued recall	Assessment of verbal memory skills.
20-minute break	
MMT-B (Part 2) Long delay free recall Long delay cued recall Recognition	Assessment of verbal recall skills.

Time

4 Results

4.1 Statistical Analysis

9 out of 10 study participants were women. The predominant incidence of female patients agrees with the results of other studies [27,33]. The mean age at onset of the disease was 34.9 (± 12.4) years, and the neurocognitive assessment was performed after an average of 8.2 (± 8.5) years of disease duration. The mean age at baseline evaluation was 43.1 (± 13.2) years. 8 patients had postoperative hypoparathyroidism following surgical intervention in the anterior neck area. The remaining 2 cases have been classified as idiopathic hypoparathyroidism. 7 study participants reported suffering from paresthesia several times a week, 6 experienced muscle cramps in arms and legs. Intestinal spasms, including stomach and colon, and myalgia were less common. One female patient reported the daily occurrence of all these symptoms.

Table 4: Characteristics of study participants.

Parameter	Study group
Number of participants	10
Gender (F:M)	9:1
Age at baseline evaluation (± SD)	43.1 (± 13.2)
Age at onset of disease (± SD)	34.9 (± 12.4)
Duration of disease in years (± SD)	8.2 (± 8.5)
Etiology	
Postoperative n (%)	8 (80 %)
Idiopathic n (%)	2 (20 %)
Acute symptoms of hypocalcemia	
Paresthesia n (%)	7 (70 %)
Muscle cramps n (%)	6 (60 %)
Intestinal spasms n (%)	2 (20 %)
Myalgia n (%)	3 (30 %)

4.2 Prevalence of Neurocognitive Dysfunction

The global MMSE score of the study group of 29.5 (± 0.8) was similar to the control group with 29.8 (± 0.4) points. None of the study participants had a score below the threshold of 24, which would indicate impaired cognitive function.

According to the MWT-B, the mean estimated IQ score of the study group was 106 (± 10.2) and therefore above the mean raw score of the norming sample, defined as 100. Both the mean raw score and the mean IQ of the study group were slightly lower than the mean scores of the control group. However, the differences are not statistically significant with a p-value of 0.06 for raw scores and 0.3 for estimated IQ.

The results of the MMT-B also revealed no significant differences between the study and control groups concerning verbal learning and memory. The statistical analysis of the test results showed no correlation between chronic hypoparathyroidism and cognitive dysfunction, as can be seen in **Table 5**.

Table 5: Comparison of raw scores of study and control group.

Characteristic	Study group	Control group	P-value
Number of participants	10	10	
Age in years (± SD)	43.1 (± 13.2)	43.3 (± 13.9)	0.85
MMSE (± SD)	29.5 (± 0.8)	29.8 (± 0.4)	1
MWT-B			
Raw score (± SD)	28.5 (± 3)	29.5 (± 4.1)	0.06
Estimated IQ (± SD)	106 (± 10.2)	111.1 (± 15.8)	0.3
MMT-B			
Total summed up list	56.9 (± 10.7)	59 (± 12.1)	0.97
Alternative list free recall (± SD)	7.1 (± 3.8)	7.5 (± 2.6)	0.94
Short delay free recall (± SD)	11.2 (± 3.2)	12 (± 3.2)	0.26
Short delay cued recall (± SD)	12.8 (± 2.7)	13.6 (± 2.4)	0.58
Long delay free recall (± SD)	12.5 (± 2.7)	12.9 (± 3.5)	0.36

Long delay cued recall (± SD)	13.4 (± 2.7)	13.4 (± 2.7)	0.72
Recognition	15.6 (± 0.9)	15.3 (± 1)	0.59

Due to the low number of male study participants, gender-specific differences could not be collected. Neither could any statements be made about how the severity and duration of the disease affect cognitive impairment.

4.3 Quality of Life (QoL)

The impact of chronic hypoparathyroidism on well-being has been reported in several studies using validated questionnaires [66,81,84]. The common clinical features like paresthesia, tetany, and seizures are mainly due to PTH-dependent hypocalcemia and demand more frequent medical consultations and longer hospital stays [80]. Rejnmark et al. noted that well-being appears to be worse in postoperative hypoparathyroidism compared to non-surgical forms [102]. This is probably since in surgical causes, the function of the parathyroid glands was impaired more abruptly.

The SF-MPQ visual analog scale measuring subjective pain intensity in the last week before the interview (whereby a score of 0 means no pain, a score of 10 means worst possible pain) documented a mean pain score of 1.4 (± 1.7) (see **Figure 5**) [103]. One patient experienced mild pain at the time of the interview, another reported moderate pain (see **Figure 6**).

Figure 5: Experience of pain in the last week before the interview. Each dot on the visual analog scale represents a patient's pain score.

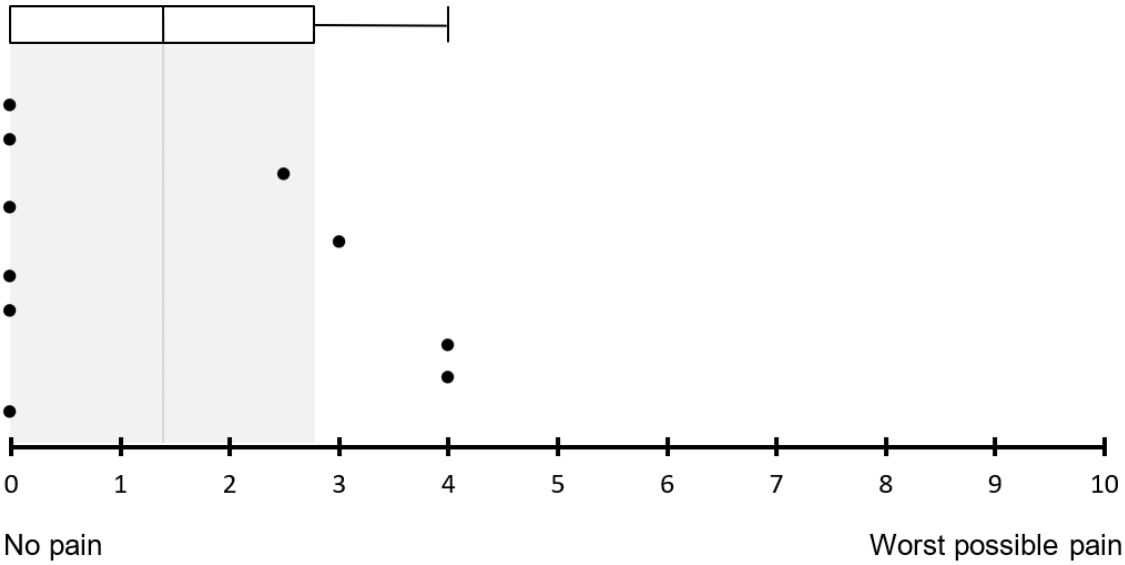
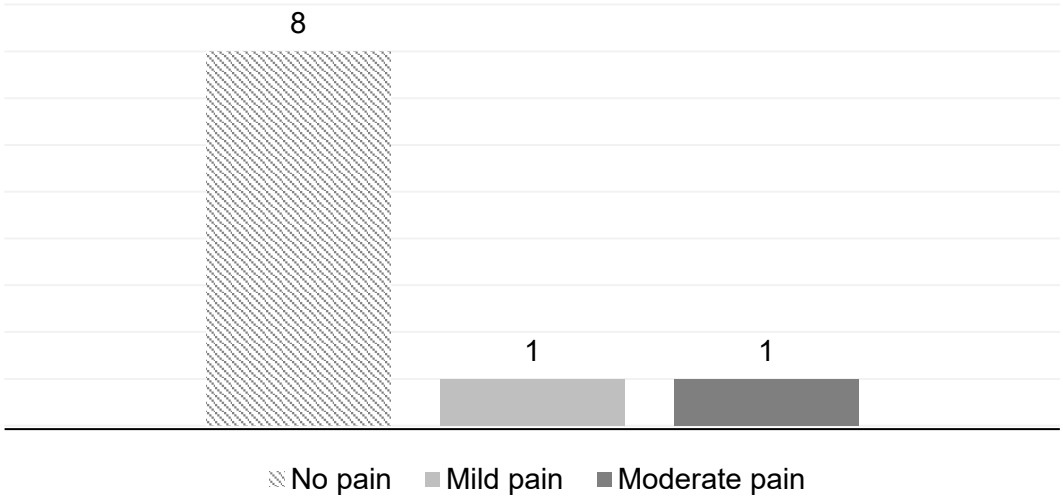


Figure 6: Experience of pain at the time of the interview.



5 Discussion

The aim of this thesis was to collect observational data on a variety of parameters and to determine the prevalence of neurocognitive dysfunction in adult patients with the rare disease chronic hypoparathyroidism.

5.1 Summary of the Results

In our 14-month recruitment process, 10 adult patients could be identified at the Medical University of Graz and underwent neurocognitive assessment. The patients were compared to 10 individuals of a control group. In 80 % of all patients, previous neck surgeries could be identified as the primary cause of hypoparathyroidism, confirming postoperative hypoparathyroidism as the most common form [20,21]. In the remaining 20 % of cases, the cause of the disease had not been determined at the time of writing, as data on possible genetic causes were not available. The 2 patients with idiopathic hypoparathyroidism were the youngest in the study population (both female; 24 and 28 years old at the time of diagnosis). The general onset of the disease was at a mean age of 34.9 (± 12.4) years, and only 2 patients were older than 45 years when the first symptoms appeared. Compared to previous studies, our study population thus developed hypoparathyroidism at a significantly younger age [35]. The neurocognitive assessment was performed in individual interviews after a mean of 8.2 (± 8.5) years since onset. 9 out of 10 patients were women. The increased incidence of hypoparathyroidism in the female gender ranging from 70 to 90 % is in concordance with other studies [27,33,34], as women are more often affected by thyroid and parathyroid diseases. A partial explanation for the higher need for neck surgery in women is suspected in the higher rates of autoimmunity and the complex interaction between thyroid and female sex hormones. Greater hormonal fluctuations due to pregnancy or menopause further complicate this.

Chronic hypocalcemia caused by PTH deficiency may develop slowly and can remain asymptomatic for years. However, clinical manifestations often include neuromuscular, cognitive, and psychiatric complications [104]. Many studies report a negative effect of hypoparathyroidism on cognitive health [56,66,80]. This statement is supported by case reports [79,105,106].

In this thesis, a neurocognitive test battery was used to explore cognitive dysfunction. No significant limitations were found using the MMSE to assess general cognitive function, the MWT-B to estimate premorbid crystallized intelligence, and the MMT-B to assess semantic verbal learning and memory. Our results showed no association between chronic hypoparathyroidism and cognitive dysfunction.

The patients most frequently suffered from paresthesias in fingers and toes (70 %) and painful muscle cramps in arms and legs (60 %). Since the onset of the disease, 3 patients have experienced myalgias and 2 patients abdominal cramps. Despite standard therapy, some patients also showed complications such as tetany (30 %) and cardiac arrhythmia (10 %) - complications that partially had to be treated with intravenous calcium administration in order to reduce neuromuscular hyperexcitability and spontaneous depolarization of nerve and muscle fibers. f

Four patients reported pain in the week before the interview, and 2 patients also experienced pain during the interview. Muscle and joint pain were most frequently described, followed by diffuse headache. No patient described their current state of health as excellent. The term brain fog was used by 2 patients to describe the presence of cognitive impairment and difficulty concentrating. The complaints about pain and the increased occurrence of brain fog reflect a reduced QoL. The impact of the disease on patients' daily routines is typically underestimated by non-exposed individuals, leading to a substantial empathy gap [107].

5.2 Interpretation of the Results

Our thesis highlights the impaired health status and well-being due to hypoparathyroidism. A decline of the patients' QoL and the most common hypocalcemic symptoms described in the literature could be observed.

However, an increased prevalence of cognitive dysfunction in patients with long-term hypoparathyroidism was not seen in our study. The low number of study participants and the young mean age at onset of disease may limit generalizability of our findings.

5.3 Limitation of Research

The search for study participants proved to be difficult because of the low incidence of this orphan disease, the COVID-19 pandemic in 2020/21 and its restrictions imposed by the Austrian government. Another limiting factor of this thesis is that no CT or MRI findings of the brain could be collected. The presence and extent of possible BGC could therefore not be determined. Furthermore, the level of education from the patients was not comparable between the study and control groups due to a lack of information.

The neurocognitive interviews lasted an average of 1.5 hours. During this time, the study participants performed exhausting cognitive work. Hypoparathyroid patients report that they tire more easily due to chronic fatigue [24]. In at least one female patient, the interview had to be briefly interrupted due to exhaustion.

5.4 Recommendations for Research

To draw valid conclusions about the link between chronic hypoparathyroidism, basal ganglia calcification (BGC), and cognitive dysfunction, CT and functional MRI scans of the brain are necessary in addition to neurocognitive/psychiatric assessment and laboratory tests. Neuroimaging evidence is needed to identify organic causes such as pathological calcification of the basal ganglia. Ectopic deposition of calcium-phosphate products damages the walls of the blood vessels in affected brain regions. This may lead to neurocognitive disturbances such as impaired memory performance and brain fog. Chronic hypoparathyroidism should therefore be considered as a rare differential diagnosis in patients with mental disorders of unknown origin.

Also, the role of PTH receptors should be taken into consideration. Type 2 PTH receptors are present in the cerebral and cerebellar cortex as well as in other areas crucial for cognition, such as the basal ganglia and thalamus. Future studies incorporating long-term PTH therapy might be helpful to understand the relationship between cognitive dysfunction and PTH [108].

The high costs of synthetic rhPTH currently allow its use only in selected cases. The goal of rhPTH therapies is to achieve normocalcemia while reducing the need for supplemental calcium and vitamin D_3 [86]. Attempts must be made to replace the common once-daily injection of rhPTH with more physiological ways of administration.

Continuous administration via infusion pumps has shown a significant reduction in urinary calcium excretion but is a very intensive form of therapy [91]. Other studies also reported improved physical and mental functioning under rhPTH therapy [84]. Due to the novelty of the rhPTH therapy, systematic and prospective studies to determine long-term effects are needed. Surgical approaches include transplantation of parathyroid tissue. One study reported normal PTH and calcium levels in the blood even 24 months after surgery [98]. However, the donor risk, the risk for infections, human leukocyte antigen (HLA) incompatibilities, and the consequences of at least short-term immunosuppressive therapy must be considered in relation to the expected benefit [97].

The effects of hormone replacement therapies using rhPTH and the surgical transplantation of parathyroid tissue remain areas for further research and require well-designed clinical trials.

6 References

1. Murray TM, Rao LG, Divieti P, Bringhurst FR. Parathyroid Hormone Secretion and Action: Evidence for Discrete Receptors for the Carboxyl-Terminal Region and Related Biological Actions of Carboxyl-Terminal Ligands. Endocrine Reviews 2005;26:78-113.

2. Khan M, Jose A, Sharma S. Physiology, Parathyroid Hormone (PTH). StatPearls [Internet]: StatPearls Publishing, Treasure Island (FL); 2020.

3. Duque EJ, Elias RM, Moysés RMA. Parathyroid Hormone: A Uremic Toxin. Toxins 2020;12:189.

4. Rejnmark L, Ejlsmark-Svensson H. Effects of PTH and PTH Hypersecretion on Bone: a Clinical Perspective. Current Osteoporosis Reports 2020.

5. Heini Murer, Nati Hernando, Ian Forster a, Biber J. Regulation of Na/Pi Transporter in the Proximal Tubule. Annual Review of Physiology 2003;65:531-42.

6. Kim K, Lee CR, Kang S-W, et al. Clinical Utility of Preoperative Vitamin D3 Injection for Preventing Transient Hypocalcemia after Total Thyroidectomy. International journal of endocrinology 2021;2021:6683089-.

7. Păduraru DN, Ion D, Carsote M, Andronic O, Bolocan A. Post-thyroidectomy Hypocalcemia - Risk Factors and Management. Chirurgia (Bucur) 2019;114:564-70.

8. Christakos S, Dhawan P, Porta A, Mady LJ, Seth T. Vitamin D and intestinal calcium absorption. Mol Cell Endocrinol 2011;347:25-9.

9. Pérez AV, Picotto G, Carpentieri AR, Rivoira MA, Peralta López ME, Tolosa de Talamoni NG. Minireview on Regulation of Intestinal Calcium Absorption. Digestion 2008;77:22-34.

10. Pike JW, Meyer MB. The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D(3). Endocrinol Metab Clin North Am 2010;39:255-69.

11. Wasserman RH. Vitamin D and the Dual Processes of Intestinal Calcium Absorption. The Journal of Nutrition 2004;134:3137-9.

12. Hernando N, Wagner CA. Mechanisms and Regulation of Intestinal Phosphate Absorption. Compr Physiol 2018;8:1065-90.

13. Clarke B. Normal bone anatomy and physiology. Clin J Am Soc Nephrol 2008;3 Suppl 3:S131-S9.

14. Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. Clin J Am Soc Nephrol 2015;10:1257-72.

15. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. American Journal of Physiology-Renal Physiology 2005;289:F8-F28.

16. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health: potential mechanisms. Nutrients 2010;2:693-724.

17. Pondel M. Calcitonin and calcitonin receptors: bone and beyond. International journal of experimental pathology 2000;81:405-22.

18. Srinivasan A, Wong FK, Karponis D. Calcitonin: A useful old friend. Journal of musculoskeletal & neuronal interactions 2020;20:600-9.

19. Bollerslev J, Rejnmark L, Marcocci C, et al. European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. European Journal of Endocrinology 2015;173:G1.

20. Marcucci G, Cianferotti L, Parri S, et al. HypoparaNet: A Database of Chronic Hypoparathyroidism Based on Expert Medical-Surgical Centers in Italy. Calcified Tissue International 2018;103:151-63.

21. Paulina Godlewska AUMBAUES-NAUJGAUBPAUMD. Risk factors of permanent hypoparathyroidism after total thyroidectomy and central neck dissection for papillary thyroid cancer: A prospective study. Risk factors of permanent hypoparathyroidism after total thyroidectomy and central neck dissection for papillary thyroid cancer: A prospective study 2020;0.

22. Bilezikian JP, Khan A, Potts JT, Jr., et al. Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. J Bone Miner Res 2011;26:2317-37.

23. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133.

24. Sinnott B. Hypoparathyroidism – Review of the Literature 2018. Journal of Rare Disorders: Diagnosis & Therapy 2018;04.

25. Villarroya-Marquina I, Sancho J, Lorente-Poch L, Gallego-Otaegui L, Sitges-Serra A. Time to parathyroid function recovery in patients with protracted hypoparathyroidism after total thyroidectomy. Eur J Endocrinol 2018;178:103-11.

26. Seo ST, Chang JW, Jin J, Lim YC, Rha KS, Koo BS. Transient and permanent hypocalcemia after total thyroidectomy: Early predictive factors and long-term follow-up results. Surgery 2015;158:1492-9.

27. Clarke BL, Brown EM, Collins MT, et al. Epidemiology and Diagnosis of Hypoparathyroidism. J Clin Endocrinol Metab 2016;101:2284-99.

28. Botto LD, May K, Fernhoff PM, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. Pediatrics 2003;112:101-7.

29. McDonald-McGinn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. Nat Rev Dis Primers 2015;1:15071-.

30. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical guidelines for managing patients with 22q11.2 deletion syndrome. The Journal of pediatrics 2011;159:332-9.e1.

31. Sullivan KE. DiGeorge syndrome/chromosome 22q11.2 deletion syndrome. Curr Allergy Asthma Rep 2001;1:438-44.

32. Vetter T, Lohse MJ. Magnesium and the parathyroid. Curr Opin Nephrol Hypertens 2002;11:403-10.

33. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Cardiovascular and renal complications to postsurgical hypoparathyroidism: A Danish nationwide controlled historic follow-up study. Journal of Bone and Mineral Research 2013;28:2277-85.

34. Zach M, Tmava-Berisha A, Schatzl S, Lehner M, Vila G, Amrein K. Die HypAus-Studie. Journal für Klinische Endokrinologie und Stoffwechsel 2018;11:45-50.

35. Abate EG, Clarke BL. Review of Hypoparathyroidism. Frontiers in Endocrinology 2017;7.

36. Hadker N, Egan J, Sanders J, Lagast H, Clarke BL. Understanding the Burden of Illness Associated with Hypoparathyroidism Reported Among Patients in the Paradox Study. Endocrine Practice 2014;20:671-9.

37. Mitchell DM, Regan S, Cooley MR, et al. Long-term follow-up of patients with hypoparathyroidism. J Clin Endocrinol Metab 2012;97:4507-14.

38. Kern M. Hypoparathyreoidismus - Retrospektive Beobachtungsstudie: Medical University of Graz; 2015.

39. Kim JH, Shin YL, Yang S, et al. Diverse genetic aetiologies and clinical outcomes of paediatric hypoparathyroidism. Clin Endocrinol (Oxf) 2015;83:790-6.

40. Weaver CM, Peacock M. Calcium. Adv Nutr 2011;2:290-2.

41. Martucci G, Bonicolini E, Parekh D, Thein OS, Scherkl M, Amrein K. Metabolic and Endocrine Challenges. Semin Respir Crit Care Med 2020.

42. Piste P, Sayaji D, Avinash M. Calcium and its Role in Human Body. Int J Res Pharm Biomed Sci 2012;4:2229-3701.

43. Aktualisierte D-A-CH Referenzwerte für Calcium. Österreichische Gesellschaft für Ernährung, 2020. (Accessed May 5th, 2020, at https://www.oege.at/index.php/wissenschaft-forschung/referenzwerte/2-uncategorised/1952-aktualisierte-d-a-ch-referenzwerte-fuer-calcium.)

44. Bringhurst FR, Demay MB, Kronenberg HM. CHAPTER 28 - Hormones and Disorders of Mineral Metabolism. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, eds. Williams Textbook of Endocrinology (Twelfth Edition). Philadelphia: W.B. Saunders; 2011:1237-304.

45. Barnett MW, Larkman PM. The action potential. Practical Neurology 2007;7:192-7.

46. Rizvi I, Ansari N, Beg M, Shamim M. Widespread Intracranial Calcification, Seizures and Extrapyramidal Manifestations in a Case of Hypoparathyroidism. North American Journal of Medical Sciences 2012;4:369-72.

47. Han P, Trinidad BJ, Shi J. Hypocalcemia-induced seizure: demystifying the calcium paradox. ASN Neuro 2015;7:1759091415578050.

48. Jesus JE, Landry A. Chvostek's and Trousseau's Signs. New England Journal of Medicine 2012;367:e15.

49. Soliman A, Saeed S, Khellah A. Clinical And Echocardiographic Effects Of Hypocalcemia Secondary To Severe Vitamin D Deficiency (vdd) And Effect Of Treatment. Archives of Disease in Childhood 2014;99:A265-A6.

50. Nijjer S, Ghosh AK, Dubrey SW. Hypocalcaemia, long QT interval and atrial arrhythmias. BMJ case reports 2010;2010:bcr0820092216-bcr.

51. Castiglione A, Odening K. [QT Interval and Its Prolongation - What Does It Mean?]. Dtsch Med Wochenschr 2020;145:536-42.

52. Vink AS, Neumann B, Lieve KVV, et al. Determination and Interpretation of the QT Interval. Circulation 2018;138:2345-58.

53. García Martín A, Varsavsky M, Cortés Berdonces M, et al. Phosphate disorders and clinical management of hypophosphatemia and hyperphosphatemia. Endocrinol Diabetes Nutr 2020;67:205-15.

54. Hyperphosphatemia. Merck Manuals Professional Edition, 2020. (Accessed May 7th, 2020, at https://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/hyperphosphatemia.)

55. Jono S, McKee MD, Murry CE, et al. Phosphate Regulation of Vascular Smooth Muscle Cell Calcification. Circulation Research 2000;87:e10-e7.

56. Aggarwal S, Kailash S, Sagar R, et al. Neuropsychological dysfunction in idiopathic hypoparathyroidism and its relationship with intracranial calcification and serum total calcium. 2013;168:895.

57. Amrein K, Scherkl M, Hoffmann M, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. European Journal of Clinical Nutrition 2020.

58. Schlögl M, Holick MF. Vitamin D and neurocognitive function. Clin Interv Aging 2014;9:559-68.

59. Aranow C. Vitamin D and the immune system. J Investig Med 2011;59:881-6.

60. Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. Journal of cellular biochemistry 2003;88:259-66.

61. Amrein K, Scherkl M, Hoffmann M, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. European Journal of Clinical Nutrition 2020;74:1498-513.

62. Prevention and Management of Osteoporosis: Report of a Who Scientific Group: Who Scientific Group; 2003.

63. Velasco PJ, Manshadi M, Breen K, Lippmann S. Psychiatric aspects of parathyroid disease. Psychosomatics 1999;40:486-90.

64. Lanciego JL, Luquin N, Obeso JA. Functional neuroanatomy of the basal ganglia. Cold Spring Harb Perspect Med 2012;2:a009621-a.

65. Wang C, Li Y, Shi L, et al. Mutations in SLC20A2 link familial idiopathic basal ganglia calcification with phosphate homeostasis. Nature Genetics 2012;44:254-6.

66. Sardella A, Bellone F, Morabito N, et al. The association between hypoparathyroidism and cognitive impairment: a systematic review. Journal of Endocrinological Investigation 2020.

67. Shoback DM, Bilezikian JP, Costa AG, et al. Presentation of Hypoparathyroidism: Etiologies and Clinical Features. The Journal of Clinical Endocrinology & Metabolism 2016;101:2300-12.

68. Donzuso G, Mostile G, Nicoletti A, Zappia M. Basal ganglia calcifications (Fahr's syndrome): related conditions and clinical features. Neurol Sci 2019;40:2251-63.

69. Zhou Y-Y, Yang Y, Qiu H-M. Hypoparathyroidism with Fahr's syndrome: A case report and review of the literature. World J Clin Cases 2019;7:3662-70.

70. Goswami R, Sharma R, Sreenivas V, Gupta N, Ganapathy A, Das S. Prevalence and progression of basal ganglia calcification and its pathogenic mechanism in patients with idiopathic hypoparathyroidism. Clinical Endocrinology 2012;77:200-6.

71. Titlic M, Tonkic A, Jukic I, Filipovic-Grcic P, Kolic K. Cognitive impairment and epilepsy seizure caused by hypoparathyroidism. Bratisl Lek Listy 2008;109:79-81.

72. Mateo D, Giménez-Roldán S. Dementia in Idiopathic Hypoparathyroidism: Rapid Efficacy of Alfacalcidol. Archives of Neurology 1982;39:424-5.

73. Verma A, Kumar A. Hypoparathyroidism presenting with late onset seizures - a report of two cases from rural India. Neuroimmunology and Neuroinflammation 2018;5:20.

74. Ogata A, Ishida S, Wada T. A survey of 37 cases with basal ganglia calcification (BGC): CT-scan findings of BGC and its relationship to underlying diseases and epilepsy. Acta Neurol Scand 1987;75:117-24.

75. Seedat F, Daya R, Bhana SA. Hypoparathyroidism Causing Seizures: When Epilepsy Does Not Fit. Case Reports in Medicine 2018;2018:5948254.

76. Kowdley KV, Coull BM, Orwoll ES. Cognitive impairment and intracranial calcification in chronic hypoparathyroidism. Am J Med Sci 1999;317:273-7.

77. Lourida I, Thompson-Coon J, Dickens CM, et al. Parathyroid Hormone, Cognitive Function and Dementia: A Systematic Review. PLOS ONE 2015;10:e0127574.

78. Usdin TB, Gruber C, Bonner TI. Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. J Biol Chem 1995;270:15455-8.

79. Kumar G, Kaur D, Aggarwal P, Khurana T. Hypoparathyroidism presenting as cognitive dysfunction. BMJ Case Reports 2013;2013:bcr2013009220.

80. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Postsurgical Hypoparathyroidism—Risk of Fractures, Psychiatric Diseases, Cancer, Cataract, and Infections. Journal of Bone and Mineral Research 2014;29:2504-10.

81. Arlt W, Fremerey C, Callies F, et al. Well-being, mood and calcium homeostasis in patients with hypoparathyroidism receiving standard treatment with calcium and vitamin D. Eur J Endocrinol 2002;146:215-22.

82. Kim SH, Rhee Y, Kim YM, et al. Prevalence and complications of nonsurgical hypoparathyroidism in Korea: A nationwide cohort study. PLOS ONE 2020;15:e0232842.

83. Hoogendijk WJG, Lips P, Dik MG, Deeg DJH, Beekman ATF, Penninx BWJH. Depression Is Associated With Decreased 25-Hydroxyvitamin D and Increased

Parathyroid Hormone Levels in Older Adults. Archives of General Psychiatry 2008;65:508-12.

84. Cusano NE, Rubin MR, McMahon DJ, et al. The Effect of PTH(1–84) on Quality of Life in Hypoparathyroidism. The Journal of Clinical Endocrinology & Metabolism 2013;98:2356-61.

85. Bohrer T, Krannich JH. Depression as a manifestation of latent chronic hypoparathyroidism. World J Biol Psychiatry 2007;8:56-9.

86. Bollerslev J, Rejnmark L, Marcocci C, et al. European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. European Journal of Endocrinology 2015;173:G1-G20.

87. Takeda Presents New Data at the Endocrine Society's 2019 Annual Meeting about the Patient Burden and Long-Term Impact of Chronic Hypoparathyroidism 2019. (Accessed March 26, 2020, at https://www.takeda.com/newsroom/newsreleases/2019/takeda-presents-new-data-at-the-endocrine-societys-2019-annual-meeting--about-the-patient-burden-and-long-term-impact-of-chronic-hypoparathyroidism2/.)

88. Porter RH, Cox BG, Heaney D, Hostetter TH, Stinebaugh BJ, Suki WN. Treatment of hypoparathyroid patients with chlorthalidone. N Engl J Med 1978;298:577-81.

89. Eller K. Hypokalziämie und Hyperkalziämie: Ätiologie, Klinik, Diagnose und Therapie. Journal für Klinische Endokrinologie und Stoffwechsel - Austrian Journal of Clinical Endocrinology and Metabolism 2014;4:40-5.

90. Tuli G, Buganza R, Tessaris D, Einaudi S, Matarazzo P, de Sanctis L. Teriparatide (rhPTH 1–34) treatment in the pediatric age: long-term efficacy and safety data in a cohort with genetic hypoparathyroidism. Endocrine 2020;67:457-65.

91. Winer KK, Zhang B, Shrader JA, et al. Synthetic human parathyroid hormone 1-34 replacement therapy: a randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. J Clin Endocrinol Metab 2012;97:391-9.

92. Winer KK, Sinaii N, Peterson D, Sainz B, Jr., Cutler GB, Jr. Effects of Once Versus Twice-Daily Parathyroid Hormone 1–34 Therapy in Children with Hypoparathyroidism. The Journal of Clinical Endocrinology & Metabolism 2008;93:3389-95.

93. Rubin MR, Sliney J, Jr., McMahon DJ, Silverberg SJ, Bilezikian JP. Therapy of hypoparathyroidism with intact parathyroid hormone. Osteoporos Int 2010;21:1927-34.

94. Mannstadt M, Clarke BL, Vokes T, et al. Efficacy and safety of recombinant human parathyroid hormone (1–84) in hypoparathyroidism (REPLACE): a double-blind, placebo-controlled, randomised, phase 3 study. The Lancet Diabetes & Endocrinology 2013;1:275-83.

95. Trupka AW, Sienel W. Die simultane Autotransplantation von Nebenschilddrüsengewebe im Rahmen der totalen Thyreoidektomie benigner Strumen. In: Siewert JR, Hartel W, editors. Digitale Revolution in der Chirurgie; 2002 2002//; Berlin, Heidelberg: Springer Berlin Heidelberg. p. 317-8.

96. Barczyński M, Gołkowski F, Nawrot I. Parathyroid transplantation in thyroid surgery. Gland Surg 2017;6:530-6.

97. Yucesan E, Goncu B, Basoglu H, et al. Fresh tissue parathyroid allotransplantation with short-term immunosuppression: 1-year follow-up. Clin Transplant 2017;31.

98. Parameswaran R, Samuel M, Satish RL, et al. Parathyroid allotransplantation to treat post-thyroidectomy hypoparathyroidism: A review of case studies. The Surgeon 2020.

99. Gluhm S, Goldstein J, Loc K, Colt A, Liew CV, Corey-Bloom J. Cognitive performance on the mini-mental state examination and the montreal cognitive assessment across the healthy adult lifespan. Cogn Behav Neurol 2013;26:1-5.

100. Creavin ST, Wisniewski S, Noel-Storr AH, et al. Mini-Mental State Examination (MMSE) for the detection of dementia in clinically unevaluated people aged 65 and over in community and primary care populations. Cochrane Database of Systematic Reviews 2016.

101. Lehrl S, Triebig G, Fischer B. Multiple choice vocabulary test MWT as a valid and short test to estimate premorbid intelligence. Acta Neurologica Scandinavica 1995;91:335-45.

102. Rejnmark L. Quality of life in hypoparathyroidism. Endocrine 2018;59:237-8.

103. Wright KD, Asmundson GJG, McCreary DR. Factorial validity of the short-form McGill pain questionnaire (SF-MPQ). European Journal of Pain 2001;5:279-84.

104. Del Rio P, Rossini M, Montana CM, et al. Postoperative hypocalcemia: analysis of factors influencing early hypocalcemia development following thyroid surgery. BMC Surgery 2019;18:25.

105. Santos VMd, Da Mata AMDF, Ribeiro KRA, Calvo ICDS. Fahr's Syndrome and Secondary Hypoparathyroidism. Romanian Journal of Internal Medicine 2016;54:63-5.

106. Terada T, Kakimoto A, Yoshikawa E, et al. The Possible Link between GABAergic Dysfunction and Cognitive Decline in a Patient with Idiopathic Hypoparathyroidism. Internal Medicine 2015;54:2245-50.

107. Derksen FAWM, Olde Hartman T, Bensing J, Lagro-Janssen A. Empathy in general practice—the gap between wishes and reality: comparing the views of patients and physicians. Family Practice 2017;35:203-8.

108. Bagó AG, Dimitrov E, Saunders R, et al. Parathyroid hormone 2 receptor and its endogenous ligand tuberoinfundibular peptide of 39 residues are concentrated in endocrine, viscerosensory and auditory brain regions in macaque and human. Neuroscience 2009;162:128-47.

7 Appendix

As part of this thesis, results were presented at the following events:

- Annual meeting of the ÖGES / OSDG / ANETS (Austrian Society for Endocrinology and Metabolism)
  April 22-24, 2021

- 28th Osteoporosis Forum of the ÖGKM (Austrian Society for Bone and Mineral Metabolism), St. Wolfgang im Salzkammergut
  October 15-17, 2020

- 22nd European Congress of Endocrinology of the ESE (European Society of Endocrinology)
  September 5-9, 2020

8 Attachments

Attachment 1: Poster for the annual meeting of the ÖGES / OSDG / ANETS (Austrian Society for Endocrinology and Metabolism), April 2021

Medizinische Universität Graz

Neurocognitive functions in chronic hypoparathyroidism

Mario Scherkl [1], Adelina Tmava-Berisha [1], Astrid Fahrleitner-Pammer [1], Karin Amrein [1]

[1] Medical University of Graz

Background

Chronic hypoparathyroidism is a rare endocrine disorder characterized by inadequately low parathyroid hormone (PTH) levels and hypocalcemia. Possible late manifestations of the consecutive calcium and phosphate disturbance are severe and may lead to intracranial calcifications and neurocognitive impairment. Affected patients report frequent brain fog, depressive symptoms, cognitive impairment, and a reduced quality of life.

Methods

In this thesis, a battery of neurocognitive tests including the Mini-Mental-State-Examination (MMSE), the Multiple-Choice-Vocabulary-Test (MWT), and the Munich-Memory-Test (MMT) was used to assess general cognitive functions. In the period from 2019 to 2021, 10 adult patients with diagnosed hypoparathyroidism could be identified as study participants and underwent neurocognitive assessment.

Results

9/10 study participants are women. The mean age at onset of the disease was 33.3 (± 9.9) years, and the neurocognitive assessment was performed after an average of 8.5 (± 8.6) years of disease duration.

The global MMSE score of the study group of 29.5 (± 0.7) was similar to the control group with 29.7 (± 0.6) points. None of the study participants had a score below the threshold of 24, which would indicate impaired cognitive function. According to the MWT, the mean estimated IQ score of the study group was 108.4 (± 9.9) and therefore above the median raw score of the norming sample, defined as 100.

The statistical evaluation of the test results showed no correlation between chronic hypoparathyroidism and neurocognitive dysfunction.

Characteristic	Study group	Control group	P-value
Participants	N = 10	N = 10	
Age in years	41.8 (± 10.2)	47.10 (± 8.0)	0.05
MMSE	29.5 (± 0.7)	29.7 (± 0.6)	0.75
MWT			
Raw score	29.3 (± 2.8)	28.7 (± 5.7)	0.71
Estimated IQ	108.4 (± 9.9)	109.9 (± 17.5)	0.40
MMT			
Short free	11.5 (± 2.5)	9.8 (± 2.2)	0.15
Short cued	12.4 (± 2.5)	9.4 (± 2.8)	0.63
Long free	12.2 (± 2.6)	9.3 (± 2.4)	0.13
Long cued	13.4 (± 2.7)	9.7 (± 2.4)	0.28

Table 1: Comparison of raw scores of study and control group

Conclusion

There is currently only limited data available on the pathophysiological link between chronic hypoparathyroidism and neurocognitive dysfunction. Further studies including functional MRI testing need to provide laboratory and neuroimaging evidence along with neurocognitive assessment to support an association between PTH deficiency and impaired cognitive functions. Also, the effects of PTH replacement should be addressed.

Attachment 2: Poster for the 28th Osteoporosis Forum of the ÖGKM (Austrian Society for Bone and Mineral Metabolism), October 2020

Medizinische Universität Graz

Klassische Epilepsie oder doch etwas anderes? Zwei Fallberichte

Tmava-Berisha Adelina[1], Lobmeyr Elisabeth[2], Urbanic Purkart Tadeja[1] , Scherkl Mario[1], Amrein Karin[1]

[1] Medizinische Universität Graz
[2] Medizinische Universität Wien

Hintergrund

Ein Hypoparathyreoidismus präsentiert sich oft atypisch, besonders bei verzögerten postoperativen Verläufen und bei nicht-postoperativer Genese. Die Calciumwerte sind meist extrem niedrig und klassische neuromuskuläre Symptome können gänzlich fehlen.

Methoden

Fallbericht einer 29-jährigen Frau und eines 3-jährigen Mädchens.

Resultate

Eine 29-jährige Patientin mit seit Jahren bekanntem M. Addison wurde nach Grand-Mal-Anfall in die Notaufnahme eingeliefert. Im Schädel-CT zeigten sich massive Verkalkungen der Basalganglien und in der Blutgasanalyse eine deutliche Hypokalzämie von gesamt 1.60 mmol/l. Zusätzlich zeigte sich bei der weiteren Aufarbeitung bei der Patientin ein beidseitiger fortgeschrittener Katarakt, der bei stark eingeschränkter Sehleistung eine rasche Operation erforderte.

Ein 3-jähriges, bisher völlig gesundes und normal entwickeltes Mädchen präsentierte sich mit einem erstmaligen Grand-Mal-Anfall. Im Schädel-CT und in der Basis-Laboruntersuchung (ohne Calcium) zeigten sich keine Auffälligkeiten. Nach mehreren Jahren mit antiepileptischer Therapie und gelegentlichen Anfällen wurde schließlich eine Elektrolytbestimmung durchgeführt, bei welcher sich eine extreme Hypokalzämie zeigte.

Nach Verbesserung der Calciumwerte durch Standard- bzw. rhPTH1-84-Therapie traten bei beiden Patientinnen keine epileptischen Anfälle mehr auf. Bei beiden konnte im Verlauf eine *AIRE*-Mutation als Ursache für den nicht-postoperativen Hypoparathyreoidismus gefunden werden (autoimmunes polyglanduläres Syndrom Typ 1, APS1).

Conclusio

Diese zwei Erstdiagnosen eines APS1 durch *AIRE*-Mutation zeigen, dass ein chronischer Hypoparathyreoidismus sich auch als Epilepsie erstmanifestieren kann. Eine Differenzierung ist nur mittels Laborwerten möglich. Eine Blutentnahme sollte daher bei erstmaligen oder unerklärlichen gehäuften Krampfanfällen zur Basisdiagnostik gehören. Die Identifikation der zugrundeliegenden Mutation erlaubt bei dem Mädchen hoffentlich eine rechtzeitige Diagnostik des voraussichtlich noch auftretenden M. Addison ohne Entwicklung einer schweren Addison-Krise.

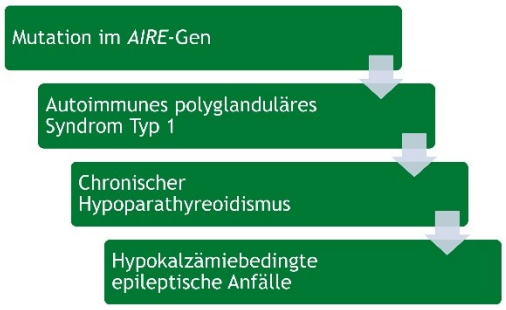


Abbildung 1: Autoimmuner Hypoparathyreoidismus

Freundliche Unterstützung von Shire, jetzt Teil von Takeda

Attachment 3: Poster for the 22nd European Congress of Endocrinology of the ESE (European Society of Endocrinology)

The HypAUS study - First results of an Austrian prospective hypoparathyroidism registry

Stefan Schatzl[1], Adelina Tmava-Berisha[2], Mario Scherkl[2], Susanne Kaser[1], Julia K. Mader[2], Astrid Fahrleitner-Pammer[2], Karin Amrein[2]

[1]Department of Internal Medicine I, Medical University Innsbruck, 6020 Innsbruck, Austria
[2]Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, 8036 Graz, Austria

Med Uni Graz

MEDIZINISCHE UNIVERSITÄT INNSBRUCK

Background

Chronic hypoparathyroidism is a hormone disorder that is typically caused by removal or damage to the parathyroid glands during or after neck surgery. Other less frequent causes include genetic and autoimmune disorders. This endocrine disorder substantially impacts quality of life for many patients because it causes a multitude of symptoms and requires chronic medication. The current standard treatment is focused on controlling symptoms by oral administration of calcium and active and native vitamin D. Recent studies have suggested positive effects of parathormone replacement therapy, but due to its high cost it is currently not often used in clinical practice.

Methods

The aim of the prospective multicentric observational HypAUS study was the establishment of a cohort of patients with chronic hypoparathyroidism, i.e. a hypoparathyroidism with a duration of more than 6 months. Between January 2017 and December 2018, 55 patients were recruited by the Medical University of Graz, the Medical University of Innsbruck, the Medical University of Vienna and three private institutes specialized in thyroid diseases. All patients have been evaluated regarding their etiology, symptoms, therapy, and quality of life. In addition to the standard examination, some subjects have undergone neurocognitive testing.

Results

At 85%, the most common cause of hypoparathyroidism is due to surgical removement, trauma or devascularization of the parathyroid glands as part of a thyroidectomy, followed by autoimmune (7%) and genetic (2%) causes.

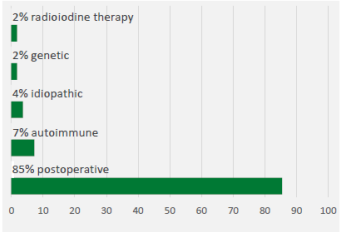


Figure 1: Most commonly observed causes

80% of the patients are female, with a median age at diagnosis of 44 years. Typical hypocalcemia-related symptoms such as paresthesia (76%) and muscle cramps (63%) were most frequently reported. A third of the patients (33%) suffered from tetany at least once since the beginning of the disease and almost every second patient (47%) needed IV calcium infusions due to pronounced hypocalcemic symptoms.

Preliminary results indicate a reduced quality of life and a tendency towards cognitive impairments, since neuropsychiatric manifestations such as depression (31%) and brain fog (22%) could also be observed.

Symptom	Prevalence
Paresthesia	42 (76%)
Muscle cramps	35 (64%)
Decreased visual functions	20 (36%)
Tetany	18 (33%)
Myalgia	18 (33%)
Depression	17 (31%)
Cardiac arrhythmia	15 (27%)

Table 1: Most commonly observed symptoms

Conclusion

The high rates of tetany and paresthesia suggest that patients with persistent – mostly postoperative – hypoparathyroidism often remain symptomatic despite current standard treatment. Individualized therapy also based on clinical symptoms with sufficient doses of calcium, cholecalciferol and calcitriol may lead to substantial improvement. We suggest that in many cases of postoperative etiology, a second hit such as radioiodine therapy or atherosclerosis leads to overt hypoparathyroidism.

Attachment 4: Infochart for patients with the results of the thesis

Infoblatt für Patienten/-innen

Ergebnisse einer prospektiven Kohortenstudie zur kognitiven Funktion bei chronischem Hypoparathyreoidismus

Das Parathormon

Der menschliche Körper hat typischerweise vier Nebenschilddrüsen im vorderen Halsbereich. Diese Nebenschilddrüsen produzieren das Parathormon, welches für die Aufrechterhaltung der Kalzium- und Phosphatspiegel im Blut notwendig ist. Beim chronischen Hypoparathyreoidismus ist die Parathormonproduktion beeinträchtigt, was in weiterer Folge zu Kalziummangel und den typischen Symptomen führt.

Ablauf der Studie

In unserer Studie wurden klinische Testverfahren angewandt, um die Auswirkungen von chronischem Hypoparathyreoidismus auf die allgemeine Gedächtnisleistung und Lern- und Merkfähigkeit zu prüfen. Hierzu wurden 10 erwachsene Patienten/-innen einer ausführlichen kognitiven Testung unterzogen. Die Ergebnisse wurden anschließend mit den Ergebnissen einer nicht erkrankten Kontrollgruppe verglichen.

Ergebnisse der Studie

Bei 80 % der Patienten/-innen trat die Erkrankung postoperativ nach chirurgischen Eingriffen im Halsbereich auf. Gründe für die Eingriffe waren meist schilddrüsenbedingte Vorerkrankungen. In den restlichen 20 % der Fälle konnte die Ursache der Erkrankung nicht eindeutig bestimmt werden und wurde deshalb als idiopathisch eingestuft. 9 von 10 Patienten/-innen waren Frauen. Das weibliche Geschlecht konnte somit mit einem höheren Erkrankungsrisiko assoziiert werden. Das mittlere Alter bei Erkrankungsbeginn betrug 35 Jahre, und die kognitive Testung wurde nach durchschnittlich 8 Jahren durchgeführt. 7 Patienten/-innen berichteten von Parästhesien (unangenehmes Missempfinden bis hin zu Taubheitsgefühlen) in den Fingern und um den Mund, die oft mehrmals pro Woche auftraten. 6 litten unter Muskelkrämpfen in Armen und Beinen. Muskelschmerzen und Krämpfe in der Magen-Darm-Region waren weniger häufig, konnten die Lebensqualität aber zusätzlich einschränken. Die statistische Analyse der kognitiven Testergebnisse zeigte jedoch keine signifikante Korrelation zwischen langjährigem Hypoparathyreoidismus und kognitiver Dysfunktion.

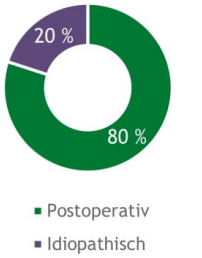


Abbildung 1: Ursachenverteilung

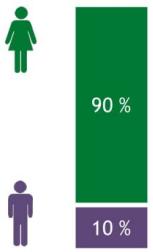


Abbildung 2: Geschlechterverteilung

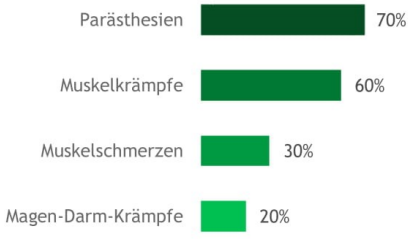


Abbildung 3: Aufzählung der häufigsten Symptome

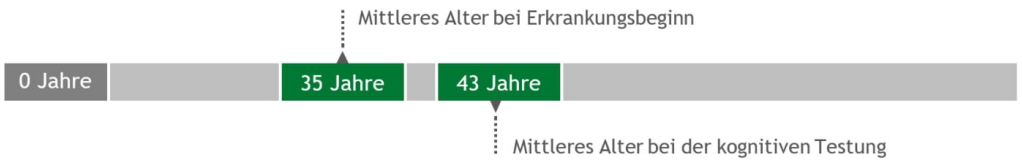


Abbildung 4: Mittleres Alter bei Erkrankungsbeginn und zum Zeitpunkt der kognitiven Testung

Attachment 5: Information for patients (Special research module)

Chronic hypoparathyroidism

Insufficiency of the Parathyroid Glands

The human body usually has four parathyroid glands, located behind the thyroid gland in the neck. These parathyroid glands produce the parathyroid hormone PTH.

Hypoparathyroidism is the rare clinical picture that occurs when the parathyroid glands do not produce enough PTH.

The term chronic hypoparathyroidism is used if the condition persists for more than six months.

Role of PTH

A hormone is a signaling molecule that transmits information to other organs via the bloodstream. PTH provides information about the current calcium and phosphate level in the blood.

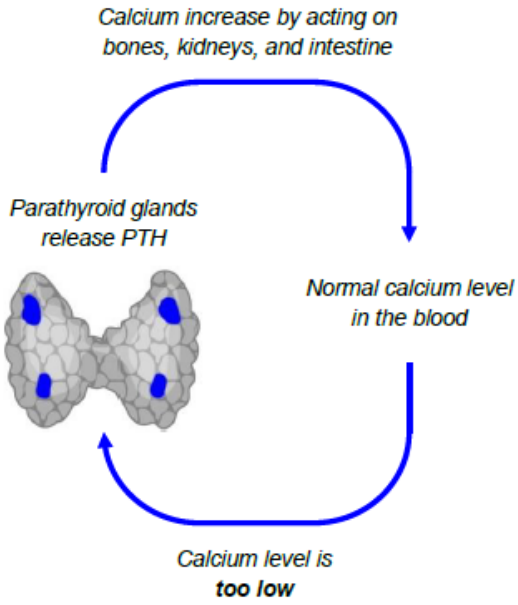
If the calcium level is too low, a condition also known as hypocalcemia, more PTH is released from parathyroid glands into the blood.

Bones, kidneys, and intestine respond to the increase in PTH and ensure that the calcium level is raised back up to normal.

Effects of PTH

So, PTH plays a key role in the regulation of normal calcium levels by acting on multiple organ systems:

- ***Bones:***
  PTH upregulates the release of calcium and phosphate from the bones into the blood.
- ***Kidneys:***
  PTH inhibits the excretion of calcium into the urine, so more calcium remains in the blood.
- ***Intestine:***
  PTH stimulates calcitriol synthesis. Calcitriol, better known as active vitamin D, enhances intestinal absorption of dietary calcium and phosphate.



Role of Calcium, Phosphate and Vitamin D

Vitamin D promotes the incorporation of calcium and phosphate into the bones and teeth, which gives them strength and stability.

About 99% of the calcium in the body is bound to phosphate and stored in the bones.

The remaining 1% of calcium regulates the contraction of muscles, nerve conduction, and the clotting of blood.

Despite its necessity for the human body, calcium cannot be produced by the organism itself and must be supplied from outside via the diet.

Consequences of Hypoparathyroidism

As a result of the inappropriately low PTH levels, the regulation of calcium and phosphate levels is disturbed, leading to abnormally low calcium, low vitamin D, and high phosphate in the blood.

If suspected, hypoparathyroidism can be diagnosed by a blood test based on these criteria.

Signs and Symptoms

The physical symptoms of hypoparathyroidism are typically due to decreased calcium levels.

Low calcium in the blood interferes with normal muscle contraction and nerve conduction, leading to abnormal sensations of the skin, muscle aches, and muscle spasms, particularly around the mouth.

Longstanding hypoparathyroidism can be associated with severe neuropsychiatric conditions including depression, cognitive impairment, movement disorders, and finally, declined quality of life.

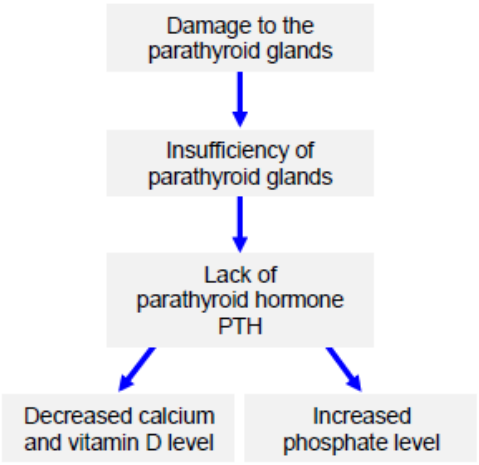
Causes of Hypoparathyroidism

The most common cause of hypoparathyroidism is damage to the parathyroid glands itself as a result of:

- surgical removement or trauma
- radioactive iodine therapy in the neck area
- radiation therapy in the neck area
- infiltration of a tumor

Further possible causes are:

- autoimmune disorders
- genetic disorders
- magnesium deficiency



Treatment of Hypoparathyroidism

Close monitoring of PTH, calcium, and phosphate levels in the blood is needed.

Initial treatment involves taking calcium and active vitamin D supplements to stabilize calcium levels. In the presence of extremely elevated phosphate levels, phosphate binders may be helpful.

A new approach is a substitution therapy in which the body is supplied with the required PTH from outside.

In chronic hypoparathyroidism, lifelong therapy may be necessary to keep the symptoms under control.

Attachment 6: Follow-up vote of the Ethics Committee

Medizinische Universität Graz
Ethikkommission

Auenbruggerplatz 2, A-8036 Graz
ethikkommission@medunigraz.at
Tel.: +43 / 316 / 385-13928, Fax: -14348

FOLGEVOTUM

gültig bis 22.12.2021

EK-Nummer:	29-062 ex 16/17
Studientitel:	HYPOPARATHYREOIDISM - prospektive cohort
Prüfer:	Assoz.Prof.Priv.Doz.Dr. Karin Amrein Medizinische Universität Graz
Sponsor:	Medizinische Universität Graz
Ansprechpartner:	Assoz.Prof.Priv.Doz.Dr. Karin Amrein, 8036 Graz, Auenbruggerplatz 2
CRO:	
Antragsteller:	Klin. Abt. f. Endokrinologie u. Stoffwechsel, Univ.-Klin. f. Innere Medizin
Ansprechpartner:	Assoz.-Prof. Priv.-Doz. Dr. Karin Amrein, 8036 Graz, Auenbruggerplatz 15

Die o.a. Studie wurde von der Ethikkommission erstmals im 'expedited Review' am 11.11.2016 behandelt. Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befangen anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: keine

Zur Beurteilung vorliegende Dokumente:

Dokumente eingegangen am 20.10.2016, begutachtet im 'expedited Review' am 11.11.2016	
✓ Antragsformular ECS	20.10.2016
✓ Originalprotokoll DA Studienprotokoll Hypopara prospektiv FINAL 1.0	18.10.2016
Informed Consent Form Informed consent Hypopara prospektiv 1.0	18.10.2016
✓ Informed Consent Form Informed consent Biobank 5.3	22.12.2015
✓ Case Report Form Case report form Hypopara 1.0 1.0	18.10.2016
✓ CV CV Max Zach 1.0	19.10.2016
✓ CV CV Gessl 19.10.16 1.0	19.10.2016
✓ CV CV Greisa Vila 1.0	20.10.2016
✓ CV CV Harald Dobnig 1.0	20.10.2016
✓ CV CV_Amrein 2016 1.0	18.10.2016
✓ CV CV Adelina Tmava 1.0	19.10.2016
Dokumente eingegangen am 29.11.2016 (in der nächsten Begutachtung mitbegutachtet)	
✓ Informed Consent Form 1.1	23.11.2016
✓ CV Mitarbeiter Vila 1.0	19.10.2016
✓ CV Mitarbeiter Tmava 1.0	19.10.2016
✓ CV Mitarbeiter Zach 1.0	19.10.2016
✓ CV Mitarbeiter Gessl 1.0	19.10.2016
✓ CV Mitarbeiter Dobnig 1.0	20.10.2016
✓ CV Mitarbeiter Amrein 1.0	18.10.2016
✓ Sonstiges: Stellungnahme zur Bearbeitungsmitteilung 1.0	23.11.2016

Dokumente eingegangen am 02.12.2016 (in der nächsten Begutachtung mitbegutachtet)	
Case Report Form 1.0	
Dokumente eingegangen am 14.12.2016 (in der nächsten Begutachtung mitbegutachtet)	
✓ Antragsformular ECS unterschrieben	14.12.2016
Dokumente eingegangen am 14.12.2016, begutachtet im 'expedited Review' am 22.12.2016	
✓ Case Report Form 1.1	23.11.2016
Dokumente eingegangen am 28.11.2017, begutachtet im 'expedited Review' am 05.12.2017	
✓ Zwischenbericht	28.11.2017
Dokumente eingegangen am 08.02.2019 (in der nächsten Begutachtung mitbegutachtet)	
✓ Zwischenbericht	08.02.2019
Dokumente eingegangen am 09.06.2019, begutachtet im 'expedited Review' am 13.06.2019	
✓ Informed Consent Form 1.2	09.06.2019
Dokumente eingegangen am 15.10.2019, begutachtet im 'expedited Review' am 31.10.2019	
✓ CV Mitarbeiter Scherkl undatiert	
Dokumente eingegangen am 19.02.2020 (in der nächsten Begutachtung mitbegutachtet)	
Zwischenbericht	10.02.2020
Dokumente eingegangen am 27.04.2020, begutachtet im 'expedited Review' am 30.04.2020	
✓ Zwischenbericht	21.04.2020
Dokumente eingegangen am 09.12.2020, begutachtet im 'expedited Review' am 17.12.2020	
✓ Zwischenbericht	27.11.2020

Datum Erstvotum: 22.12.2016

Die Ethikkommission geht - rechtlich unverbindlich - davon aus, dass es sich um keine klinische Prüfung nach AMG bzw. MPG handelt.

Es handelt sich um eine Studie im Rahmen einer Diplomarbeit.

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen

- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen

- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)

- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

zusätzliche Auflagen: Die behördlich vorgeschriebenen Maßnahmen hinsichtlich der COVID-19 Pandemie müssen beachtet werden. Der Prüfer und der Sponsor müssen in ihrem jeweiligen Wirkungskreis unter allfälliger Beachtung von Leitlinien gewährleisten, dass keine zur Bekämpfung der Pandemie benötigten Ressourcen gebunden werden bzw. ausreichend Personal vorhanden ist und die TeilnehmerInnen durch ihre Studienteilnahme keiner zusätzlichen Infektionsgefahr ausgesetzt werden.

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. UG 2002. Information: Mitteilungsblatt der Universität. UID: ATU 575 111 79. Bankverbindung: Raiffeisen Landesbank Steiermark IBAN: AT443800000000049510, BIC: RZSTAT2G

Graz, 17. Dezember 2020

Univ.Prof.DI Dr.Josef Haas
Vorsitzender

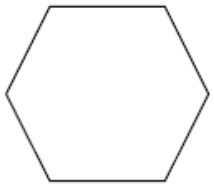
Univ.Prof.Dr.Hans Dimai
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

Medizinische Universität Graz, Auenbruggerplatz 2, A-8036 Graz. www.medunigraz.at
Rechtsform: Juristische Person öffentlichen Rechts gem. UG 2002. Information: Mitteilungsblatt der Universität. UID: ATU 575 111 79. Bankverbindung: Raiffeisen Landesbank Steiermark IBAN: AT443800000000049510, BIC: RZSTAT2G

Attachment 7: Collected patient data

A prospective cohort study of cognitive function and depression in hypoparathyroidism



Name:

Geburtsdatum:

Familienstand:

Beruf:

Größe/Gewicht:

Zeitpunkt Erstdiagnose des Hypoparathyroidismus:

Komorbiditäten:

Medikamente:

Attachment 8: Case report form, Version 1.1

HYPOPARATHYREOIDISMUS – prospektive Studie Version 1.1, 23.11.2016

ID: Center/Nr./Initialen: _ _ _ _/ _ _ _/ _ _ (1st patient in Graz: GRA001MH) Date: _ _/ _ /201_ (dd/mm/yyyy) Investigator: _ _ (Max Huber: z.B. MH)

- Familienstand: □ ledig □ in einer Partnerschaft □ verheiratet □ geschieden □ verwitwet
  (für Frauen: Geburten: _ _ _ _ _ _ _ _ Schwangerschaften _____)
- Beruf u. Pensum: _
- Reduktion des Berufspensums bzw. krankheitsbedingte Invalidität seit Krankheitsbeginn:
  □ nein □ ja (Falls zutreffend: Höhe der Reduktion bzw. Invalidität) _ _ _ _ _ _ _ _ _ _
- Alter bei Erkrankungsbeginn: _
- Ätiologie (Erkrankungsursache):
  - □ postoperativ (z.B. nach Schilddrüsen-Operation)
  - □ autoimmun im Rahmen von APS Typ 1
  - □ autoimmun (ev. mit nachgewiesenen Antikörpern)
  - □ nach Radiojodtherapie
  - □ genetisch (DiGeorge-Syndrom)
  - □ idiopathisch

---

1. **Lebensqualität (QoL): SF-12**

Bitte beantworten Sie die grau unterlegten Fragen, indem Sie bei den Antwortmöglichkeiten die Zahl ankreuzen, die am besten auf Sie zutrifft.

	Ausge-zeichnet	Sehr gut	Gut	Weniger gut	schlecht
1. Wie würden Sie Ihren Gesundheitszustand im Allgemeinen beschreiben?	1	2	3	4	5

Im folgenden sind einige Tätigkeiten beschrieben, die Sie vielleicht an einem normalen Tag ausüben.			
Sind Sie durch Ihren derzeitigen Gesundheitszustand bei diesen Tätigkeiten eingeschränkt? Wenn ja, wie stark?	Ja, stark eingeschränkt	Ja, etwas eingeschränkt	Nein, überhaupt nicht eingeschränkt
2. **mittelschwere** Tätigkeiten, z.B. einen Tisch verschieben,	1	2	3

Seite 1 von 7

Staubsaugen, Kegeln, Golf spielen			
3. **mehrere** Treppenabsätze steigen	1	2	3

Hatten Sie in den vergangenen 4 Wochen aufgrund Ihrer körperlichen Gesundheit irgendwelche Schwierigkeiten bei der Arbeit oder anderen alltäglichen Tätigkeiten im Beruf bzw. zu Hause?	Ja	Nein
4. Ich habe **weniger geschafft** als ich wollte	1	2
5. Ich konnte **nur bestimmte Dinge** tun	1	2

Hatten Sie in den vergangenen 4 Wochen aufgrund seelischer Probleme irgendwelche Schwierigkeiten bei der Arbeit oder anderen alltäglichen Tätigkeiten im Beruf bzw. zu Hause (z.B. weil Sie sich niedergeschlagen oder ängstlich fühlten?)	Ja	Nein
6. Ich habe **weniger geschafft** als ich wollte	1	2
7. Ich konnte nicht so **sorgfältig** wie üblich arbeiten	1	2

	Überhaupt nicht	Ein bisschen	Mäßig	Ziemlich	Sehr
8. Inwieweit haben die Schmerzen Sie in den vergangenen 4 Wochen bei der Ausübung Ihrer Alltagstätigkeiten zu Hause und im Beruf behindert?	1	2	3	4	5

In diesen Fragen geht es darum, sie Sie sich fühlen und wie es Ihnen in den vergangenen 4 Wochen gegangen ist. (Bitte kreuzen Sie in jeder Zeile die Zahl an, die Ihrem Befinden am ehesten entspricht). Wie oft waren Sie in den letzten 4 Wochen...	Immer	Meistens	Ziemlich oft	Manch-mal	Selten	Nie
9…. ruhig und gelassen?	1	2	3	4	5	6
10…. voller Energie?	1	2	3	4	5	6
11…. entmutigt und traurig	1	2	3	4	5	6

	Immer	Meistens	Manchmal	Selten	Nie
12. Wie häufig haben Ihre körperliche Gesundheit oder seelische Probleme in den vergangenen 4 Wochen Ihre Kontakte zu anderen Menschen (Besuche bei Freunden, Verwandten usw.) beeinträchtigt?	1	2	3	4	5

2. Therapie

Woraus besteht Ihre derzeitige Therapie des Hypoparathyreoidismus? (wenn zutreffend, welche Dosierung?)

- □ Calcitriol (z.B. Rocaltrol „Roche“) _
- □ Calcium (z.B. Calcium „Sandoz“) _
- □ Vitamin D (z.B. Oleovit D3-Tropfen) _
- □ Thiazid-Diuretikum (Monopräparat z.B. Esidrex bzw. in Kombination mit anderen Wirkstoffen zur Therapie einer Herzinsuffizienz z.B. Concor plus) _
- □ Teriparatid (Forsteo) _
- □ Magnesium_ _
- □ andere Präparate _

---

3. Symptome eines Kalziummangels (Hypokalziämie)

Leiden Sie zurzeit bzw. haben Sie seit Beginn der Erkrankung an einem der folgenden Symptome gelitten? (Falls zutreffend: wie oft bzw. wie lange?)

- □ Parästhesien (Brennen, Kribbeln, Ameisenlaufen, Taubheitsgefühl, Nadelstichgefühl, Kälte-/Wärmemissenmpfindung) _ _ _ _ _ _ _ _ _ _ _ _ _ _
- □ Muskelkrämpfe (z.B. an Armen, Beinen, etc) _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _
- □ Krämpfe des Verdauungstraktes _
- □ Myalgien (Muskelschmerzen) _

---

4. Akute Komplikationen im Rahmen einer Hypokalziämie

Kam es bei Ihnen seit Erkrankungsbeginn jemals zu einem der folgenden Symptome? (Falls zutreffend: wie oft? Wurde deswegen der Notruf verständigt bzw. eine Notfallambulanz aufgesucht)

- □ Tetanie (Krampfanfall) _
  Notruf bzw. Notfallambulanz: □ ja □ nein

- □ Laryngo- bzw. Bronchospasmus (Krampf der Stimmritze bzw. der Bronchien)
  _
  Notruf bzw. Notfallambulanz: □ ja □ nein

□ Episoden mit ansonsten unerklärlicher Atemnot und/oder pfeifender Atmung

_ _

Notruf bzw. Notfallambulanz: □ ja □ nein

□ Herzrhythmusstörungen (Herzklopfen, Herzstolpern, Schwindel, Synkope/Bewusstseinsverlust) _

Notruf bzw. Notfallambulanz: □ ja □ nein

Wurde Ihnen im Rahmen einer Hypokalziämie jemals Calcium i.v. (als Infusion) verabreicht? (wenn zutreffend, aufgrund welcher Symptomatik?)

□ nein

□ ja _

---

5. Kardiovaskuläre Symptome u. Erkrankungen

Trat seit Erkrankungsbeginn eines der folgenden Symptome auf bzw. wurde diagnostiziert? (Falls zutreffend: wie oft bzw. Zeitpunkt der Diagnose und Therapie?)

□ Hypotonie und Synkopen (niedriger Blutdruck, RR <100/60 mmHg; oft zusammen mit Schwindel, Kollapsneigung/Synkopen, Herzrasen, Kopfschmerz, rasche Ermüdbarkeit, Blässe, Konzentrationsmangel)

_ _

□ QT-Zeitverlängerung bzw. Herzrhythmusstörung im EKG (oft mit allgemeinem Unwohlsein, Herzrasen, Herzklopfen, Brustenge, Synkopen, Schweißausbruch)

_ _

---

6. Neuropsychiatrische Manifestationen

Ist Ihnen seit Erkrankungsbeginn eines der folgenden Symptome bzw. Krankheitsbilder an Ihnen aufgefallen bzw. wurde diagnostiziert?
(Falls zutreffend: wie lange bestehen die Symptome bzw. Zeitpunkt der Diagnose und Therapie?)

□ Depression bzw. depressive Verstimmung _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Angststörung _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Psychose _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Bewegungsstörungen:

□ Parkinson-artig (erhöhte Muskelspannung, Bewegungsarmut, Muskelzittern)

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Chorea-artig (unwillkürliche Bewegungen, v.a. Finger und Gesicht)

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Brain fog (verminderte Konzentrations- und Erinnerungsleistung)

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Dysphagie (Schluckstörungen) _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Ataxie (Störung von kontrollierten Bewegunsabläufen bzw. der Körperhaltung beim Stehen, Gehen oder Zeigen auf Gegenstände)

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

□ Dysarthrie (Störung der Sprachbildung und der Sprachmotorik bei uneingeschränktem Lese-, Schreib- und Sprachverständnis)

_ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _ _

---

7. Sehleistung

Hat sich Ihre Sehleistung seit Erkrankungsbeginn verschlechtert? (Falls zutreffend: wurde von einem Facharzt eine Ursache diagnostiziert, z.B. Papillenödem, Katarakt/grauer Star?)

□ nein

□ ja _

---

8. Nieren und Harnkalzium

Sind bei Ihnen seit Erkrankungsbeginn Nierensteine aufgetreten bzw. diagnostiziert worden? (Falls zutreffend: wie oft bzw. Therapie?)

□ nein

□ ja _

Wurde bei Ihnen seit Erkrankungsbeginn ein erhöhtes Harnkalzium im Spontanharn bzw. 24 Stunden-Harn festgestellt?

□ nein

□ ja _

Wurde bei Ihnen seit Erkrankungsbeginn eine Nephrokalzinose (Verkalkungen des Nierengewebes) diagnostiziert? (Falls zutreffend: welche Art der Bildgebung?)

□ nein
□ ja _____

---

9. Krankenhausaufenthalte

Befanden Sie sich seit Erkrankungsbeginn in stationärer Behandlung? (Falls zutreffend: wann bzw. Grund des Aufenthalts?)

□ nein
□ ja _____
_____
_____
_____

---

10. Infektionen

Sind Sie seit Erkrankungsbeginn an Infektionen erkrankt (mit oder ohne daraus resultierender Antibiotikatherapie)? (Falls zutreffend: welche?)

□ nein
□ ja _____
_____

---

11. Frakturen

Haben Sie seit Erkrankungsbeginn Knochenbrüche erlitten? (Falls zutreffend: wieviele bzw. Unfallhergang?)

□ nein
□ ja _____
_____

Kam es im Laufe einer oder mehrerer Frakturheilung/-en zu einer verzögerten Frakturheilung? (keine bzw. verminderte Knochenneubildung an der Bruchstelle in den ersten 4 Wochen nach der Fraktur)

□ nein
□ ja _____

12. Knochendichte

Wurde bei Ihnen seit Erkrankungsbeginn eine Knochendichtemessung (Osteodensiometrie durch DXA; z.B. an Lendenwirbelsäule oder Hüfte) durchgeführt? (Falls zutreffend: Werte bzw. Befund vorhanden?)

□ nein

□ ja _

---

13. Craniale Bildgebung

Wurde bei Ihnen seit Erkrankungsbeginn eine craniale Bildgebung durchgeführt (Computertomografie/CT oder Magnetresonanztomografie/MRT des Kopfes; Falls zutreffend: wurden intrakranielle Kalkablagerungen diagnostiziert?)

□ nein

□ ja _

---

14. Zahnstatus

Kam es bei Ihnen seit Erkrankungsbeginn zu Zahnausfällen oder Problemen bei zahnärztlichen Eingriffen? (z.B. Implantatlockerung, etc)

□ nein

□ ja _

---

15. Andere subjektiv oder objektiv relevante Probleme

Gibt es andere mit der Erkrankung assoziierte Probleme, die nicht in diesem Fragebogen behandelt wurden?

_ _

_ _

_ _