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Mineral Deficiencies

Electrolyte Disturbances, Genes, Diet and Disease Interface

Edited by Gyula Mózsik and Gonzalo Díaz-Soto

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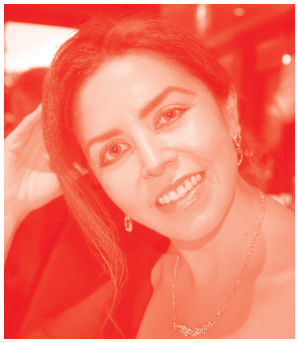

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Contributors
Andre Luis Maion Luis Maion Casarim, Ayotunde Oladunni Oladunni Ale, Oluwayomi Samson Akande, David Da Rocha-Afodu, William F. Simonds, Fong-Fu Chou, Jin-Bor Chen, Gyula Mozsik, Gonzalo Díaz-Soto, Sananda Mondal, Debasish Panda, Kahiu Ngugi, Jane Cheserek, Chrispine Omondi, Nyshadham S.N. Chaitanya, Sibani Sahu, Syed Arsalan Akhter Zaidi, Rahul Bollam, Kainat Saleem

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Meet the editors

Gyula Mózsik, MD, Ph.D., ScD (med), is Professor Emeritus of Medicine at the First Department of Medicine, Univesity of Pécs, Hungary. He was head of this Department from 1993 to 2003. His specializations are medicine, gastroenterology, clinical pharmacology, clinical nutrition, and dietetics. His research fields are biochemical pharmacological examinations in the human gastrointestinal (GI) mucosa, mechanisms of retinoids, drugs, capsaicin-sensitive afferent nerves, and innovative pharmacological, pharmaceutical and human dietary nutrition. He has published about 360 peer-reviewed papers, 197 book chapters, 19 monographs, 692 abstracts, and has edited 37 books and given 1120 regular and review lectures. He has also organized thirty-eight national and international congresses and symposia. He founded the "International Conference on Ulcer Research" (ICUR), International Union of Pharmacology, Gastrointestinal Section (IUPHAR-GI SECTION), Brain-Gut Society symposiums, and Gastrointestinal Cytoprotective symposiums. Dr. Mózsik received the Andre Robert Award from the International Union of Pharmacology-GI Section in 2014. Fifteen of his students have been appointed as full professors in Egypt, Cuba and Hungary.

Dr. Gonzalo Díaz-Soto is an Associate Professor of Endocrinology and Nutrition at Valladolid University Medical School, Spain. He received his MD and master's in Bioscience in Experimental Endocrinology on Calcium Sensing Receptor. He completed his specialization in Endocrinology and Nutrition at the Hospital Clinic of Barcelona. His Ph.D. was focused on normocalcemic hyperparathyroidism. He has completed his Ph.D. program with a clinical internship in Endocrinology Services of the Hôpital Bicêtre and Institute Gustave Roussy, Paris, France. He currently works at the Hospital Clínico Universitario de Valladolid, Spain, in the Endocrinology, Diabetes and Nutrition Department. His research areas and published articles focus on endocrinology, especially the thyroid, parathyroid and diabetic disorders.

Contents

Preface

There is a close association between food and health. Metals, inorganic compounds, and their elements that act as cofactors for enzymes that play an essential role in the various biological processes constitute mineral nutrients. During biological processes, inorganic metals are transformed into mineral (organic) forms. Written by international experts from India, Taiwan, Kenya, Nigeria, the United States, and Brazil, this book, over eight chapters, focuses on "mineral deficiencies" in patients with different diseases. The book offers important information for agricultural experts, chemists, biochemists, physicians, internists, surgeons, human clinical nutritionists, and food industry experts.

The editors thank the contributors for their excellent work and cooperation during the preparation of this book. The editors are also especially thankful for the excellent support of Ms. Romina Rovan, Author Service Manager at IntechOpen.

Gyula Mózsik
University of Pécs,
Hungary

Gonzalo Díaz-Soto
University of Valladolid,
Spain

Section 1

Introduction

Chapter 1

Introductory Chapter: Mineral Deficiencies - Electrolyte Disturbances, Genes, Diet and Disease Interface

Gyula Mózsik and Gonzalo Diaz Soto

1. Introduction

This book, *Mineral Deficiencies - Electrolyte Disturbances, Genes, Diet and Disease Interface*, discusses mineral deficiencies in human nutrition. It is a follow-up to our previous book, *Nutrition in Health and Disease - Our Challenges Now and Forthcoming Time* [1], which detailed problems of malnutrition, starvation, economics, and society across four continents.

2. Early histological backgrounds of "scientifically approach to nutrition"

For more than 200 years, the fibre in plant foods has been known by animal nutritionists to have significant effects on digestion. Its role in human nutrition began to be investigated towards the end of the nineteenth century. Denis Burkitt (1911–1993), a surgeon, brought back to the United Kingdom from Africa ideas from a range of disciplines along with his own observations to propose a radical view of fibre in human health. Later, Burkitt met with three physicians in London, Cleave, Campbell, and Trowell, who collected observations on fibre consumption and the distribution of different diseases in Africa. Trowell worked in London at St. Thomas' Hospital and had visited Uganda in 1948, 1958, and 1970, and Kenya in 1929. He observed that the distribution of different diseases changed along with changes in the population during the observed periods. Namely, the numbers of different diseases increased in people who came from Western countries, whereas the numbers remained unchanged in the African populations. In addition, he noted the emergence of entirely new diseases like diabetes mellitus, hypertension, and malignant conditions in the population coming from Europe.

Burkitt built on the work of Cleave, Campbell, and Trowell to develop the "fibre hypothesis" [2, 3], the main point of which is that fibre consumption decreases the risks of obesity, diabetes, dental caries, various vascular disorders, large bowel cancer, appendicitis, and diverticulosis, particularly in the UK population. This hypothesis was considered groundbreaking at the time, and spurred further research (e.g., "Dietary Fibre in Europe. Current State of Knowledge on Definitions, Sources, Recommendations, Intakes and Relationships to Health." Nutrition Research Reviews. 2017;**30**:149-190).

3. Problems in nutrition research from 1970 to the present

3.1 Dietary fibre

The chemical compositions of fibres of different origin have been widely studied, as have their different behaviours such as viscosity, linkage capacity, detoxication abilities, and changes before vs. after treatments like heating, boiling, cooking, and so on [4].

To understand the actions of different foods, we must investigate their effects in healthy subjects as well as subjects with different diseases. For this, written and permitted protocols in accordance with guidelines from different national and international authorities and permission from persons who participate in the studies are required [4].

3.2 Metabolic wards in nutrition research

To correctly measure the actions of foods (given either orally or parenterally), the correct methodology must be used. As such, controlled dietary studies typically take place in metabolic wards. These wards must be fully equipped with the appropriate measuring instruments, staff (e.g., physicians, laboratory assistants, etc.), and study participants. Details of different accepted methods can be found in our previous book [5].

3.3 Chemicals and drugs in food

One of the biggest problems with foods for human consumption is the wide and systematic use of chemicals (plant origin food) and drugs such as hormones (animal origin foods). The aim of these systematic treatments is to increase crop and livestock yields.

My research team studied the effects of capsaicin (from paprika) on the so-called capsaicin-sensitive afferent nerves and compared its anti-inflammatory properties with those of nonsteroidal anti-inflammatory drugs (NSAIDs), which can produce mucosal damage, bleedings, ulceration, and of course stomach pain. Our results showed that paprika for human consumption contains anywhere from one to eight different pesticide residues (see The Report Card: Pesticides in Sweet Bell Peppers at www.ewg.org; [6], pp. 152–153).

3.4 Controls of our observations

We present the results of our population observations along with lists of agricultural chemicals used both in plants and animals, although we do not identify the individual treatments.

4. Conclusion

The field of nutrition is very wide, thus collaboration is necessary to investigate the different problems stemming from plant and animal foods before their arrival to humans. We must identify the main problems of industrial food factories, food storage, and more.

We all know that the world's population is growing exponentially, whereas the world's food supply is dwindling. Thus the possibility of malnutrition (starvation) is extremely high.

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Our responsibility lies in the production of better foods for the prevention and treatment of different diseases. We must work together and learn from one another, focusing our attention on problems of agriculture, climate, economics, medicine, and more.

Author details

Gyula Mózsik[1*] and Gonzalo Diaz Soto[2]

1 First Department of Medicine, University of Pécs, Hungary

2 University of Valladolid, Spain

*Address all correspondence to: gyula.mozsik@gmail.com

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Section 2

Interface of Gene-Diet Disease

Chapter 2

Nutrigenomics: An Interface of Gene-Diet-Disease Interaction

Sananda Mondal and Debasish Panda

Abstract

Healthy diet and proper nutrition are basic necessity of life and play a key role in preventing diseases. Nutrigenomics (NG) is an emerging approach in nutritional research which deals with the gene-diet interactions. The concept of nutrigenomics is not new and it is commonly associated with "inborn errors of metabolism", the rare genetic (inherited) disorders in which the body cannot properly turn food into energy. These disorders are related to insufficient availability of metabolic enzymes or cofactors due to alteration of gene. Usually cure of these diseases lies in restricted diet. Presently non communicable diseases (NCDs) like cardiovascular diseases, obesity, diabetes and cancers are outnumbering the other health ailments among the different human populations of world. The main reason behind the occurrence of these NCDs is the abruptly changing life style and food habits after industrial revolution. With the advent of industrial revolution and economical concerns, the life style of people across the world has changed so much so that it resulted in approximately millions of death cases due to these NCDs. Study related to NG is one step forward in nutritional research involving the techniques of nutrition, molecular biology, genomics, bioinformatics, molecular medicine and epidemiology together to understand the role of food as an epigenetic factor which unravel its role in the occurrence of these diseases. Hence, under the prevailing scenario of world health, it has become an urgency to boost NG research to find cure for dreaded diseases caused due to lack of healthy food and improper nutrition. Thus, such type of research findings ensures the effective benefit of genomic revolution for mankind near future.

Keywords: nutrigenomics, non communicable diseases, personalized nutrition, human health, genomic study

1. Introduction

Life, as a single-cell embryo, which is literally an envelope of the human diploid genome primed for replication. Almost every cell of a multicellular organism contains the same type of genetic material—its genome. Chromosomes, nucleic acid molecules that are the repository of an organism's genetic information, are the largest molecules in a cell and may contain thousands of genes as well as considerable tracts of intergenic DNA. This genome has to be replicated with high fidelity millions of times during development to a fetal and adult stage and millions of times thereafter simply to replenish dead cells and cells lost as a result of exfoliation. Many cofactors and substrates are required for DNA replication and DNA repair. Any error during proof reading of DNA may lead to faulty

replication, accumulation of such errors may further trigger cell death by apoptosis. Consequently, there is an accumulation of mutations at the base sequence or chromosomal level as a result of genotoxic insults due to endogenous and exogenous factors is now recognized as a fundamental underlying cause of developmental defects and accelerated aging as well as of an increased risk of degenerative conditions such as infertility, immune dysfunction, cancer, and cardiovascular and neurodegenerative diseases [1–5].

Dietary reference values (DRVs) provide us a guide for the appropriate intake of nutrients for prevention of diseases caused by deficiency (e.g., scurvy in the case of vitamin C deficiency) or excess (e.g., iron-overload disease, which may be fatal in excess iron in the cell system) [6]. It is important to determine these extreme conditions associated with nutritional disorders now-a-days and the biggest challenge lies in the prevention of these type of developmental and degenerative disease in populations which are not short of food, fortified food, or supplements but needs intervention through appropriate intake of micronutrients individually or in combination (nutriomes) to optimize cellular and organism performance on both a personal and a genetic subgroup level at different life stages. Optimization of cellular function ultimately depends on the prevention of damage to the nuclear and mitochondrial genome [7–9].

1.1 Nutrigenomics

Nutrigenomics, an globally emerging high-throughput science which depicts the effect of genetic variation in response to diet. The term "nutrigenomics" was first given by Peregrin [10] and after one year it was reviewed by Van Ommen and Stierum, [11]. In a molecular era, Wellen and Hotamisligil, [12], considered nutrients as "signalling molecules" which transmit and translate the dietary signals into the cell and within the cellular system it changes the expression of genes in nucleus leads to changes in protein and metabolite expression. Now the big question arises that what is happening within the cell system when we are having our meals in less or excess amount? So to get the answer of this question we have to study in detail of food-gene interlinking signaling mechanism which is the science behind nutrigenomics.

The sciences of nutrigenetic and nutrigenomic are based on three central factors i.e. firstly, there is a great inherited genomic diversity between the ethnic groups and individuals affected by nutrient bioavailability and its metabolism. Secondly, people may differ greatly in their food habit/nutrient availability and choices depending on cultural, geographical, economical, and taste perception differences. Thirdly, malnutrition (deficiency or excess) itself can affect gene expression and genome stability [13].

The field, nutrigenomics involves multiple disciplines under one umbrella to the study the designing of individual's diet that leads to stability of genomes by minimizing the DNA damage, epigenome alterations (DNA methylation), transcriptomics (i.e. RNA and micro-RNA expression), proteomics (protein expression) and finally metabolomics i.e. controlled metabolite changes. Study of all the field individually and interlinking of all is very important.

Within nutrigenomics, the studies related to nutrient-gene interaction and its potential for both intra- and transgenerational effects is epigenetics [14, 15]. In genetics, epigenetics is the processes which control the expression of certain genes by up/down regulating without altering the DNA sequence, whereas the reversible changes of gene expression in epigenetics is due to DNA methylation, histone modification and chromatin-associated proteins which controls the expression of house-keeping genes and suppress the expression of parasitic DNA such as transposons. However,

epigenomics is the study which deals with the analytical part of complete epigenetic changes takes place on a genome in a cell/entire organism. Epigenetic processes strongly influenced the normal growth and development of an organism. The epigenomic changes can be inherited upto 2 to 3 generation, which is modified by diet.

In a nutshell, the study of nutrigenomics requires a collaborative effort to protect the human population from endangered diseases by maintaining the balance in genetics and the industries of public health, food science and culinary. It's very easy task to make a tasty food by putting some lard or butter in it, and it's going to be tasty and yummy. But the whole population have to accept the challenge that how to prepare good tasty healthy food without using much oil or butter or ghee or any kind of unhealthy food product which is not good for health. By observing the present trend of increasing lifestyle disease, personalised nutrition diet chart should be prescribed based on individuals genomic construction by the nutritionist and this will be the future aspect of nutrigenomics. This chapter has emphasized on the nutrigenomics approach based on gene-diet interaction in relevance to existing advance studies to understand its present and future prospect, and how to protect human population from such non-communicable diseases?

2. Dietary signals and nutrient sensors

Dealing with the complex human genome, nutrigenomics has the ability to decipher variability of genome in terms of wide range of nutrient concentration and a variety of food nutrition by identification of specific dietary signal, signal sensing or perceiving receptors. Ruden et al., [16] did an nutrigenomics research, an experiment with Drosophila which is model organism and depicted that each and every nutrient have numerous targets sites with various affinities and specificities. It was found that, drosophila has adipose-like tissues and a lipid transport system, which has a similarity with humans in respect to obesity and associated diseases than any other model organisms. In addition, Müller and Kersten [17] recognized specialized cellular-sensing mechanisms and considered nutrients and dietary metabolitesas signaling elements. The molecular structure of the nutrients are naturally designed in such a way that it carries the information that how to activate a specific signaling pathways to hit the target site. Minor changes in structure (e.g., saturated vs unsaturated fatty acids or cholesterol vs plant sterols) can have a profound influence on which sensor pathways are activated. Its a great challenge for the scientist to identify the molecular pathways and the up/downstream regulation by each nutrients. Study of nutrigenomics can allow the identification of molecular pathways by genome-wide characterization of nutritional target genes. This type of information can help the researchers to understand the plan of action of individual nutrient and how it is linked with diet which has an important role in good health and diseases. Ultimately, nutrigenomics research will lead to development of evidence-based healthful food and lifestyle advice and dietary interventions for contemporary humans.

For instance, Patsouris et al., [18] revealed that though the role of PPARα towards obesity is unclear then also there is some clue where PPARα has some important function in obesity-linked pathophysiology of type 2 diabetes. Recently, it has been demonstrated that PPARα directly regulates expression of genes involved in hepatic gluconeogenesis and glycerol metabolism [18, 19]. Visceral obesity is linked to increased free fatty acid levels [20], elevated levels of free fatty acids in the cytosol promote the plasma free fatty acids to binds with the PPARα, and these molecules may be recognized by the liver as "hunger" or "in need of glucose" signals resulting in increased gluconeogenesis in a PPARα-dependent manner, particularly under conditions of hepatic insulin resistance.

Mandard et al., [19], Kersten et al., [21] reported that fasted PPARα null mice mutant (lack of functional PPARα) suffers from a variety of metabolic defects, include hypothermia, hypoglycemia, hypoketonemia, and elevated plasma-free fatty acid levels.

3. Nutrigenomic diseases and biomarkers

Research regarding nutrigenomics is based on the principal of individuals nutrition-gene-disease interaction and how to protect the mankind from endangered non communicable diseases (NCDs) like cardiovascular diseases, obesity, diabetes, respiratory diseases, metabolic syndrome and cancers globally? Such type of NCDs are mediated by exposure of particular food components chronically, these are basically busy junk food eating lifestyle diseases of cities. These kinds of nutritional disorder are detected by biomarkers. It may be some disturbed lipid profiles to check the levels of cholesterol and/or triglycerides, increased blood pressure, or abnormal sensitivity of insulin as indicator of NCDs, like cardiovascular disease or metabolic syndrome. These biomarkers are mainly single proteins or metabolites or certain body functions that leads to be an detector for proteomics and metabolic changes in individuals body may be a causative agent's of a variety of chronic diseases which depends on the particular individuals genotype. The molecular aspects of individuals DNA damage can be diagnosed by a number of complementary ways are as follows: (i) damage to single bases (e.g. DNA adducts such as the addition of a hydroxyl radical to guanine caused by oxidative stress); (ii) abasic sites in the DNA sequence (measurable by use of the aldehyde-reactive probe); (iii) DNA strand breaks (commonly measured using the Comet assay); (iv) telomere shortening (measured by terminal restriction fragment length analysis, quantitative PCR or flow cytometry); (v) chromosome breakage or loss (usually measured using micronucleus cytome assays or metaphase chromosome analysis), and (vi) mitochondrial DNA damage (usually measured as deletions or base damage in the circular mitochondrial DNA sequence). These use of damaged DNAs as a biomarkers were recently validated at various levels based on the nutrient associated evidence (cross-sectional epidemiology and intervention studies) and disease (cross-sectional epidemiology and prospective cohort studies) as reported by Fenech, [8]. The micronucleus assay in cytokinesis-blocked lymphocytes is currently the best validated biomarker for nutritional genomic studies of DNA damage. In addition, a well validated nutrigenomics tool is transcriptomics, it includes the microarray assay to analyze the mRNA copies for all actively transcribed genes. The advantage of this technique is within same time it can analyze the expression level of transcripts, thousands of genes in a single assay. In peripheral blood cells, studies of gene-expression patterns have been shown to be specific for diseased states. Whereas, Martin et al., [22] noted that disease-specific gene-expression patterns in blood cells have been identified for breast tumors and leukemia was revealed by Valk et al., [23], and those patterns now used as biomarkers for the detection of diseases.

4. Gene-diet-disease interaction

SNPs (Single nucleotide polymorphism) or SNVs (single nucleotide variants) are most widely acceptable markers now a days, responsible for genetic variation. Genotypic variations can be detected by SNPs or SNVs, and we can prescribe proper diet plan to avoid non communicable diseases (NCDs) like cardiovascular diseases,

obesity, diabetes and cancers. In this context, Ramos-Lopez et al., [24] revealed the sweet taste receptor (TAS1R2) related to taste perception and Ramos-Lopez et al., [25] depicted cluster of differentiation 36 (CD36), were associated with dyslipidemia in the peoples of Mexico, consume high amounts of carbohydrates and fats respectively. In addition, common variants of genes which regulate homocysteine metabolism, like methylene tetra hydrofolate reductase (MTHFR) and methionine synthase (MTR), are linked with the increased risk for breast cancer among individuals who intakes lower amount of folate, vitamin B6, and vitamin B12 [26]. The status of Vitamin D show polymorphism among the population and have the ability to modulate various metabolism in the organism [27]. Interestingly, the SNPs of the vitamin D receptor (VDR) gene, affect the availability of vitamin D [28, 29], and results osteoporosis in postmenopausal women with low calcium intakes [30]. Moreover, SNPs in genes encoding lipid proteins such as apolipoprotein C3 (APOC3) and apolipoprotein A1 (APOA1) conferred a higher risk of metabolic syndrome in subjects with a Western dietary pattern [31, 32]. Likewise, an increased risk of hypertension and CVD was observed with moderate and heavy coffee drinkers which was associated a genetic variation in the cytochrome P450 family 1 subfamily A member 2 (CYP1A2) gene [33, 34]. Additionally, studies using genetic risk scores (GRS) have been examined the cumulative effect of SNPs on diet interactions and susceptibility of diseases. Macronutrient is having the ability to modify the obesity GRS with greater values of adiposity [35]. Furthermore, obesity GRS interacted with the intake of sugar-sweetened beverages [36], and fried food consumption [37] in relation to BMI and obesity in several cohort studies.

4.1 Nutrigenomics and obesity

Obesity is a chronic low-grade nutrition related inflammatory disorder and the important factor which is associated with a group of metabolic abnormalities/comorbidities commonly includes insulin resistance and hyperinsulinemia, hypertension, impaired glucose tolerance, noninsulin-dependent diabetes mellitus cardiovascular disease (CVD), type 2 diabetes, and a number of cancers [38, 39]. For the progression of obesity and the associated comorbidities are resultant of abnormal lifestyle leading habits, so here is the perfect place where nutrigenetics and nutrigenomics contribute their work to minimize obesity. Now the question which can be raised that is; whether all the individuals or populations are affected with obesity if obesogenic environment is provided; the answer is no, it is based on genetic variability and interaction with environmental factors according to Nakamura et al., [40], Nettleton et al., [41], Reddon et al., [42]. With reference to this the obesogenic environment comprises dietary nutrients, age, gender, ethnicity, duration of sleep, amount of physical activity, sedentary behavior, stress, smoking, alcohol consumption, use of medication, and depression. So it is an conclusive evidence that environmental factors is the primary cause for obesity vis gene-nutrient-disease interaction. If an individual having a good dietary habit with specific timing throughout the day along with physical exercise of at least 30 min daily, then he/she can avoid such kind of metabolic disorder and its comorbidities. Nutrigenomics explain us the complex interactions of genome and its regulation differences among the obese phenotype that vary both within and across populations [43–46].

However, Hill et al. [47] given a concept positive energy balance which include increased intake of energy, decreased energy output and results deposition of energy. In this concept energy is represented as calories, if the intake of energy from diet is greater than the output then it cause (i) resting metabolic rate, (ii) absorption and metabolism of dietary nutrients, (iii) heat production or thermogenesis, and (iv) physical activity, a state of positive energy balance results to promote

deposition of triacylglycerol within adipose tissue. Likely, in vice-versa condition, a state of negative energy balance results to promote lipolysis of triacylglycerol and mobilization of fatty acids from adipose tissue.

In addition, Stockard [48] described a fact that environment has an immense impact in obesity. 100 years ago he stated that the embryo and fetus develops in mother's womb can show a dramatic variation in the phenotype without changing the genomic constitution of the offspring while providing a moderate environmental constraint during specific periods of time in the development of the embryo. Along with this findings a new concept is explains the science working behind this i.e. epigenetic changes associated with obesity. Goldberg et al., [49] demonstrate the prenatal and early postnatal periods have a critical role in the developmental induction of obesity. Here, the epigenetics performed the lead role, during early nutritional environment of the fetus can increase the susceptibility to develop obesity in later life. Epigenetics can induce a heritable changes in gene expression without altering the gene sequences, it is basically the integral regulating and determining factor of when and where specific genes are expressed. The detail methylation pattern of epigenetics was depicted by Bird, [50], he noted that methylation at the 5′position of cytosine in DNA within a CpG (cytosine and guanine nucleotides linked by phosphate) dinucleotide is very common in mammalian genomes and leave a stable epigenetic mark which is transmitted through DNA replication and cell division. This de novo methylation is catalysed by DNA methyltransferases (Dnmts) 3a and 3b, and maintained through mitosis by gene-specific methylation of hemimethylated DNA by Dnmt1 [51].

Furthermore, different experiments done by various scientist describes versatile experiences related to obesity. Some evidential facts revealed by nutrigenomic scientists that if a new born with lower birth weight means the baby have reduced fat mass. Infants with lower birth weight who undergo early catch-up growth which is characterized by greater accumulation of fat relative to lean body mass have an increased risk of becoming obese in later life compared with those born at higher birth weights [52–54]. Similarly, in another experiment Singhal et al., [55], Singhal, [56] reported that infants born with lower birth weight having an catch-up growth were fed formula milk show increased risk of cardio-vascular disease in later life. A number of studies revealed that there is a greater chance of incidence of obesity in adults who were fed formula milk as compared to breast fed during infancy [57, 58], but exception are also there who were not fitted into this condition [59].

4.2 Nutrigenomics and cardiovascular disease (CVD)

Cardiovascular disease (CVD), basically a heart disease affecting the heart and blood vessels includes arteries, capillaries, and veins. The CAD disease includes atherosclerotic, coronary and ischemic heart diseases, individuals carrying such diseases having plaques throughout the inner walls of arteries and leads to heart attacks. While age, gender and genomic constitution are an immutable risk factors for the occurrence of CVD, modifiable risk factors play a major role in the causation and progression of the disease. Some of the extra risk factors which is very common now a days due to busy and stressful life schedule like hypertension, hyperlipidemia, obesity, diabetes, atherosclerosis, thrombosis and smoking is a causative agent of CVD. Analyzing the complexity of the etiology of CVD outlined here Juma et al., [60] narrate some dietary recommendation for CVD prevention based on individuals genetic constitutions. In nutrigenomics diet is considered as environmental factors and which has a direct relationship with the development of chronic diseases, the CVD is not aloof of that. It's a well established fact proved by scientists that the personalized diet composition has a strong risk factor for development of CVD [61–64].

Obesity, itself is a curse in human population and is a leading risk factor to develop cardiovascular disease, diabetes mellitus and a number of cancers which is already discussed. In this context, to maintain the energy balance several polymorphic genes are involved to control the development of CVD in certain "favorable" or "unfavorable" condition [65]. Moreover, Lusis, [61] describes the role of atherosclerosis in the pathogenesis of CVD, it constitutes the key element and can be regarded as a complex combination of lipid transport and metabolism disorder with chronic inflammation. The levels of total cholesterol, LDL cholesterol, and triglycerides elevated permanently in the blood plasma which is causative agent for the development of atherosclerotic plagues, whereas increased levels of high density lipoprotein (HDL) i.e. cholesterol showed a protective role [65]. Genes responsible for encoding the apolipoproteins can be regulated by some signaling agents like hormones and enzymes but, it show differential sensitivity in population to develop cardiovascular diseases. In this context, individuals carrying the allele E4 of the apolipoprotein E gene show higher low-density lipoprotein-cholesterol (bad cholesterol) levels with increased intake of dietary fat as compared to those who carrying the E1, E2 andE3 alleles having equivalent amounts of dietary fat [66]. AG to A transition in the promoter of APOA1 gene is associated with increased HDL-cholesterol concentration but the results across studies are not consistent [67]. Whereas, Ordovas et al. [68] found that the allele A was associated with the decreased serum HDL levels. The genetic effect was reversed, however, in women who ate more polyunsaturated fatty acids (PUFA). In men, this type of fat effect was significant when alcohol consumption and tobacco smoking was considered in the analysis. Also specific polymorphism in genes encoding lipid transport proteins, their receptors, and lipid-processing enzymes and inflammation related proteins were shown to be associated with the characteristic changes in blood lipid concentrations [69–73].

To prevent or treat CVD an intense debate/discussion has been taking place for best dietary plan where the composition of macronutrients, the percentage of total fat along with different fatty acids presents are important [74–79]. Likewise, the source or origin of diet's composition is very essential, for e.g., individuals taken monounsaturated fatty acids from olive oils are different from monounsaturated fatty acids intake from meat and other foods of animal origin [79, 80]. Similarly, there is lots of controversy over the best origin and type (omega-6 and omega-3 series) of polyunsaturated fatty acids (PUFA) as reported by Jakobsen et al., [79] and Russo, [81] for prevention or treatment of CVD. In a same platform and same type of case study done by Shai et al., [82] and Sacks et al., [83] for controlling body weight and cardiovascular related risk factors where emphasis is given on to take high carbohydrate, low fat diet in comparison to high fat, low carbohydrate diet. In 1965, Keys et al, [84] in their study stated that it was an individual's "intrinsic characteristics" which controls the effect of diet in plasma concentrations of cholesterol, is an variable factor for person to person. Based on the facts, nutrition related counseling focused on weight reduction and normalization of lipid profiles through diet, exercise, and medication for the prevention of CVD.

4.3 Nutrigenomics and Diabetes mellitus

Diabetes mellitus (DM), a group of metabolic diseases, results from defects in insulin secretion and insulin activity or both which is characterized by hyperglycemia. Georgoulis et al. [85] reported that due to this metabolic disease DM, various organs like blood vessels, heart and kidneys are dysfunction and/or failure, and now a days this disease is considered a global burden [86]. The International Diabetes Federation's recent estimates indicate that 8.3% of adults (382 million

individuals) have diabetes, and the number of individuals with this disease is expected to rise beyond 592 million in less than 25 years [86]. DM fall into two broad etiopathogenetic categories: type 1 and type 2 DM known as T1DM and T2DM, respectively. The epidemic global obesity noted by Prentice [87], he stated that virtually T2DM will be a major health issue in the world create a major drain on health budgets. Individual with obesity increases the risk of developing the disease DM by at least 10 fold as compared to normal one [88, 89]. In developing countries, peoples are shifted their lifestyle from traditional diets system to modernized fast food eating habit which include frequent consumption of red meat, refined carbohydrates and saturated fats is leads to obesity [90]. The insulin hormone, which is an important controller of glucose and fat metabolism is secreted from β-cells of pancreas. Irregular secretion insulin is observed in both the cases i.e. obesity and T2DM. Glucolipotoxicity is the results of high sugar and saturated fatty acid in diet on regular basis as suggested by Prentki et al. [91] and it negatively controls the secretion of insulin of from the β-cells, and results hyperglycemia and hyperlipidemia.

Flavonoids present in the diets include flavones, flavonols, flavanones, isoflavones, and anthocyanins. Various experimental studies suggested the protective role of polyphenols on glucose homeostasis mechanism, along with this some specific molecules like flavanols, luteolin, quercetin and others have a great impact on different steps of intracellular signalling pathways (insulin secretion, insulin signalling and glucose uptake, enhancing mitochondrial status, suppression of inflammatory cytokine production and reactive oxygen species (ROS)/reactive nitrogen). In addition to flavonoids, phenolic acids and tannins also have inhibiting property of the enzyme α-glucosidase and α-amylase which is responsible for carbohydrate digestion [92–97]. For instance, Song et al. [98] noted that consumption of apple or tea was associated inversely with T2DM risk. This is in accordance with the Health Professionals Follow-Up Studies also suggested that higher consumption of anthocyanins, particularly from pears, apples and blueberries, were inversely associated with T2DM [99].

Caffeic acid, chlorogenic acid (present in coffee) and ferulic acid (esterified to hemicelluloses in cereals) are the most common phenolic acids [96]. An inverse result with caffeinated, decaffeinated coffee and caffeine intake with T2DM in a dose-response manner (1–6 cups/day), compared with no or rare coffee consumption was observed in different epidemiological studies, which support the hypothesis i.e. habitual coffee consumption is associated with a substantially lower risk of T2DM [100, 101].

Resveratrol (trans-3,5,4′-trihydroxystilbene) is a natural phenol act as an phytoalexins, found in the skin of grapes, blueberries, mulberries, raspberries, peanutsand red wine, helps in reducing the complications of diabetes in many organs and tissues including liver and pancreatic β-cells and in different diabetic animal models [102]. It also improve the glucose homeostasis and give protection to pancreatic β-cells. It has an important role in insulin secretion and amelioration of metabolic disorders [103].

Whereas, Afzal et al., [104] depicted that lower vitamin D levels represent a risk factor for incident of T2DM in humans. However, the levels of hypovitamin D along with increased levels of parathyroid hormone (PTH) is an independent predictor of β-cell dysfunction, insulin receptor and glycemia [105]. Patients with T2DM with established hypovitaminosis improve glycaemia and insulin secretion by Vitamin D replenishment, not only through a direct action on pancreatic β-cell function but also via regulation of plasma calcium levels, which regulate insulin synthesis and secretion [106, 107].

4.4 Nutrigenomics and cancer

Cancer is a multiple stages process in which gene expression, and protein and metabolite function begin to run aberrantly [108]. In today's genomic era, the cellular events which intercede the activation of carcinogenesis upon modulation by dietary factors, has led to flow of significant information which helped in understanding of this disease [109]. Cancer susceptibility may increase due to inherited mutations in genes. Gene diet interaction may increase the risk of developing cancer. Endogenous reactions, such as oxidations or from exogenous agents, sunlight exposure (skin cancer), such as tobacco smoke (lung cancer), aflatoxin (liver cancer), and relatively high doses of ionizing radiations (many types of cancers) [110] induces cancer.

It is mandatory to have communication between nutrition, metabolism, and gene expression for upholding body homeostasis. Human genome and nutrition jointly interacts to do the same. Individual's health condition and susceptibility to disease may get affected due to this [111]. Nutrient regulates transcription factors at the molecular level which then modifies the gene expression (up or down), consequently to adjust the metabolic responses [112].

Diet is a blend of protective, carcinogenic, and mutagenic agents all together and are metabolized by the enzymes of biotransformation process. Risk of developing cancer can be modified through genetic polymorphisms that change protein expression or the function of these enzymes. Foods ingested by humans are proposed to contain more than 25,000 [113]. Role of different bioactive food ingredients in cancer pathogenesis has been studied and found that, among these, more than 500 types of bioactive food ingredients is proved to be possible predisposing agents.

For carcinogens, diet is considered as a source (intrinsic or cooking-generated) present in certain foods or constituents acting in a protective manner (vitamins, antioxidants, detoxifying enzyme-activating substances, etc.) [114]. Carcinogen metabolism affecting polymorphisms may modify probability of contact between carcinogens and target cells, thus acting at the stage of cancer initiation [65].

In hormone dependent tumors such as breast, prostate, ovarian and endometrial cancers, influences of polymorphisms of gene encoding factors involved in hormonal regulation are most strongly manifested. Polymorphisms in sex hormone receptor genes comprising those encoding estrogen receptors, progesterone receptor, and androgen receptor have been shown to be associated with cancer risk modulation [65]. Hormonal regulation can be influenced on interaction with dietary factors. Obesity has strongly impact on hormonal status. Apparently, some food components, such as phytoestrogens are known to be processed by the pathways similar as sex hormones [115].

There are various examples of the effects of diet on cancer risk. High consumption of red meat increase the risk of colorectal cancer [66]. N-Acetyl transferase (NAT) exists in two forms: NAT1 and NAT2, it is a phase II metabolism enzyme. Several polymorphisms exist in NAT1 and NAT2, some of them are capable of slow, intermediate, or fast acetylations. Heterocyclic Aromatic amines found in heated products like cooked red meat get through acetylation by NAT. On cooking of muscle meat at high temperature, some amino acids may react with creatinine to generate heterocyclic aromatic amines (HAA). Acetylation activates HAA to reactive metabolites which bind DNA and cause cancers. This acetylation can only be performed by NAT2 fast acetylators. People who consumed relatively large quantities of red meat with NAT fast acetylator genotype had a higher risk of developing colon cancer in them [66].

Specific dietary irritants, such as salts and preservatives have been suggested as being carcinogens for gastric cancer [116]. C667T polymorphism in MTHFR gene

which reduces enzymatic activity is inversely associated with occurrence of colorectal cancer. Less consumption of folate, vitamin B12, vitamin B6 or methionine in diet are associated with increased risk for cancer in CC or TT phenotype of MTHFR gene [117].

It has also been found that reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals attack DNA bases, resulting in potential mistranscription of DNA sequence [118]. Such disruptions can interfere with DNA replication and thus produce mutations in oncogenes and tumor suppressor genes. ROS can also result in breakage of DNA strand, resulting in mutations or deletions of genetic material [119].

Dietary fibers have a protective effect against bowel cancer [120]. Growth of colonic tumors in both *in vitro* and *in vivo* systems gets inhibited on consumption of fish oil which is rich in omega-3 fatty acids [121–123].

Fruits and vegetables rich in bioactive components can prevent carcinogenesis by several mechanisms such as blocking metabolic activation through increasing detoxification. Detoxification enzymes as flavonoids, phenols, isothiocyanates, allyl sulfur compounds, indoles, and selenium can be modulated on consuming plant foods [124, 125].

Some of these bioactive components which may influence carcinogen metabolism, cell signaling, cell cycle control, apoptosis, hormonal balance and angiogenesis are calcium, zinc, selenium, folate, vitamins C, D and E, carotenoids, flavonoids, indoles, allyl sulfur compounds, conjugated linoleic acid and N-3 fatty acids [126]. Bioactive ingredients which play protective role in the cancer development are lycopene from tomatoes, resveratrol from grapes and berries, numeric acid from cinnamons, hesperidin from citrus fruits, carotenoids from red vegetables and fruits, ascorbic acid, coffee acid from coffee, types of soluble fibers, polyunsaturated and fatty acids from marine animals have [127]. Intake of proper diet with sufficient minerals and vitamins which are involved in regulatory and enzymatic processes reduces the risk of cancer. The deficiency of these micronutrients may lead to abnormalities. For example, zinc and folate is involved in DNA repairing process. Further natural compounds from plant source like apigenin (celery, parsley), curcumin (turmeric), epigallocatechin-3-gallate (green tea), resveratrol (red grape, peanuts, and berries), genistein (soybean), and allyl sulfur (garlic) have been reported to affect the cell cycle by different mechanisms. Some of these changes may be associated with the processing of synthesized proteins at the post-translational level like shifts in the phosphorylation process of the main regulatory factors of cell division [128]. Tumor behavior can also be changed by other food ingredients through accelerated cell death and enhanced apoptosis. Apoptosis occur through two known pathways: the intrinsic, mitochondrial-mediated pathway; and the extrinsic, death receptor-mediated pathway [129].

Many of the studies by American Cancer Society [130] have shown the reduced risk of cancer associated with consumption of foods rich in vitamin C, such as fruits and vegetables. On contrary, evidence indicates that vitamin C supplements do not reduce cancer risk. From the above finding it can be said that, activity of fruits and vegetables in preventing cancer is due to consumption of many vitamins and other phytochemicals in a combination, not due to vitamin C alone.

5. Conclusion

This chapter deals with the role of nutrigenomics for the prevention of non-communicable diseases. The mother nature has made all humans almost genetically similar but, only 0.1% variation makes one individual unique from others with

respect to their phenotype and individual susceptibility to disease or health and also their differing response to nutrients. Interestingly, same diet can be a risk factor for some individuals whereas in others, it may prove beneficial. Besides, some diets may regulate genes to help in maintenance of health whereas, Some of them act as possible inducer of disease. Thus, based on knowledge of individual nutritional requirements, nutrition status and genotype; personalized nutrition & diet recommendations can be made to maintain the balance between health and disease to offer a healthy life. Major challenge is, in a populous country like ours, where people are still fighting for their basic needs, personalized nutrition system approach is a dream. Now, even the rural India is not spared from this menace of non communicable disease as it has also started embracing city culture, post era of globalization and urbanisation has brought significant changes in eating habits of rural India as well. At this moment, the most pertinent question is how to overcome this public health concern. The nutrigenomics approach is most effective and the only way out but on contrary it is not going to be so cheap to be available for masses. Also it is a very difficult task to handle the huge population with nutritional intervention as it will require adequate qualified professional along with advance lab facilities. For the time being as an alternative, public health awareness programme can play an important role in different way to protect the people from these diseases in broader sense. It shall basically focus on early identification of at-risk individuals and appropriate intervention in the form of weight reduction, changes in dietary habits and increased physical activity to help to prevent, or at least delay the onset of dietary disorders until India build itself capable in all respect to implement fully functional individual nutrigenomics approach.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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Author details

Sananda Mondal* and Debasish Panda
Department of Crop Physiology, Institute of Agriculture, Visva-Bharati, Sriniketan 731236, West Bengal, India

*Address all correspondence to: mondalsananda@gmail.com

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Chapter 3

Mineral Deficiencies: A Root Cause for Reduced Longevity in Mammals

Nyshadham S.N. Chaitanya and Sibani Sahu

Abstract

Metals, inorganic compounds and their elements that act as cofactors for enzymes that play an essential role in various biological processes constitute mineral nutrients. Their primary source is soil and enters the climax consumers in food chain through plants as they contain most minerals that are essential for humans. They are required in small and precise amounts according to their requirement they were classified as Major (phosphorous (P), potassium (K)), Secondary (calcium (Ca), magnesium (Mg), sulphur (S)), Minor/trace/rare (Boron (B), chlorine (Cl), chromium (Cr), fluoride(F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), sodium (Na), vanadium (V) and zinc (Zn)). The daily requirement of minerals for individuals for effective biological function inside the cell is known as recommended dietary allowance (RDA) that varies for element. The daily requirement of major element is up to 10 g/d, whereas secondary and micro minerals was 400 - 1500 mg/d and 45 µg/d - 11 mg/d, respectively. Meats, vegetables, fruits, grains contains high amount of minerals that protect humans from mineral deficiencies. Some of the mineral deficiencies include ageing, cancer, hair loss etc. The key for these root problems include supplementation of healthy foods rich in minerals and understanding the importance of food by nutrition education, practice of physical activity, and about food habits. A detailed understanding of each mineral and their biological importance through mechanism of action studied in detail to overcome their deficiencies.

Keywords: nutrients, recommended dietary allowance, ageing, food habits, health

1. Introduction

A regulated diet with all the constituents consumed in appropriate way maintains cell homeostasis and keeps the body under physiological state that are essential for cellular demands. A number of factor contribute to body function such as biomolecules, vitamins, minerals, and hormones etc.....of these minerals gain utmost importance due to availability inside the cell is low but shows a major effects even small change in concentration. Minerals perform wide variety of functions, which are essential for existence of organism. Some of them form integral components, some as cofactors, and some as essential components of enzymes. The existence of these minerals as part of enzymes helps to play a role in metabolism of molecules

consumed through diet and maintain cell homeostasis. Some of the minerals acts in concert with aid of hormones according to their need in specific organelle. Minerals either in part or in combination with vitamins shows major functions required for the cell and their deficiencies shows adverse side effects although not hereditary. Minerals classified according to the need includes major (phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S)), minor/trace/ rare (Boron (B), chlorine (Cl), chromium (Cr), fluoride (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), sodium (Na), vanadium (V) and zinc (Zn)). In this chapter a detailed explanation of selected minerals about their importance as a source requirement, uptake and transport mechanism, toxicity and tolerance mechanism, taken as means of measurement for determining their beneficial effects to study in detail about the specific role in metabolism their mechanism of action and deficiency diseases associated with reduced life span had described.

Decline of physiological functions leading to senescence of cells with arrest at G1 phase is characteristic feature of ageing [1]. At cellular level senescence was caused due to several factors such as oxidative stress, mitochondrial dysfunction, inflammation, autophagy deregulation, telomere shortening [2, 3]. Cells senesce either due to continuous replication or due to stress induction thereby activating p16, p53 pathways and phosphorylation of Rb protein [4] leading to inflammatory condition with high lysosomal β-galactosidase activity [5–7]. As cells continuously, divide chromosomes containing telomere with repeated nucleotides region gets shortened [8] leads to replicative senescence [9] and result in ageing. In humans, the repeated sequence at telomere region is TTAGGG [10]. Cells capable of replicating continuously express telomerase for replication of telomere ends of chromosome, which had tendency to reverse ageing process and used as targeted approach [2]. Increased ROS production due to stress apart from normal cellular homeostasis as a compensatory mechanism aggravates ageing phenomenon. Free-radical theory proposes ROS leads to oxidative damage and contributes to plays a role in the ageing process [11]. First call to increased ROS levels inside the cells is activation of survival pathways, which further leads to apoptosis due to failure of antioxidant system to defence against ROS that ultimately leads to cell death [12, 13]. Several factors were responsible for production of ROS that disturbs balance between cell survival and cell death through increased redox potential towards pro-inflammatory state and connects oxidative stress, inflammation and ageing [14–16]. The release of pro-inflammatory agents inside the senescent cells include TNF-α, IL-6, IL-1β [17] regulated by transcription factors such as AP-1, NFκB [18]. The activation of AP-1, NFκB requires kinases such as ERK, JNK, p38MAPK, PI3K [19] and leads to expression of target proteins such as MMP9, ICAM-1, iNOS, COX-2 [20–22]. Mitochondria apart from playing a role in oxidative phosphorylation system it also plays a role in apoptosis, metabolism, innate immunity and ageing [23–25]. Mitochondrial regulation occurs through PGC-1 (α & β) that responds to NAD+ levels inside the cell [26, 27] and in response to SIRT1 regulation occurs by HIF-1α independent of PGC-1 [28]. In ageing NAD+ levels decreases without loss of SIRT1 but downregulates it [29]. One of the contributing factor for cell survival under stress conditions is autophagy [30]. Autophagy is downregulated under nutrient rich conditions through mTOR protein [31] and stimulated through AMPK by phosphorylating mTOR (inactivation) ultimately activating ULK-1 [32]. Reports reveal autophagy deregulates due to overexpression of mTOR [33, 34] in ageing. Several Genetic events (mTOR, TGFβ), Molecular events (oxidative stress, autophagy) also contribute to ageing phenomenon. A summary of factors responsible for cellular ageing were shown in **Figure 1**.

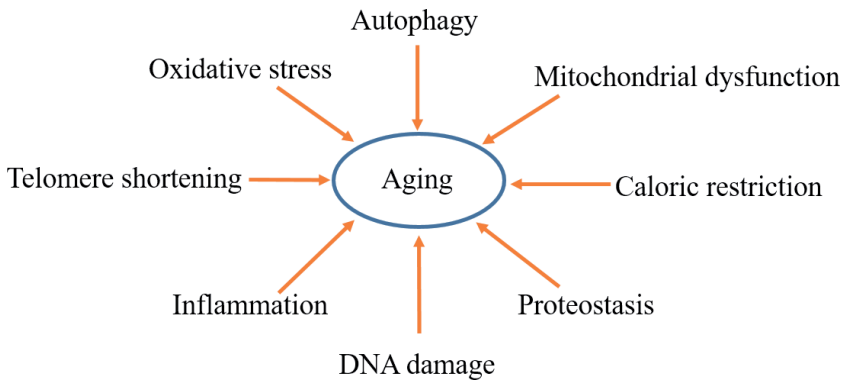

Figure 1.
Factors responsible for aging: Different factors enhances process of aging includes autophagy, oxidative stress, shortening of telomere, caloric restriction, proteostasis, inflammation, mitochondrial dysfunction and DNA damage.

2. Deficiency of major mineral elements and lifespan

2.1 Phosphorous (P)

Phosphorous is mostly present in meat, fish, eggs, and milk and dietary intake is 0.8-1.0 g/day. Phosphorus is essential for the formation of healthy bones, part of buffer system and component of DNA and RNA. Functions of phosphorous include formation of high-energy phosphates, nucleic acids, nucleotide coenzymes. Activation of enzymes require phosphate moiety and found in cell walls. Phosphorus deficiency include rickets, osteomalacia observed mostly in cases of malnutrition, anorexic individuals, or alcoholics. Symptoms are poor appetite, anxiety, and irritability. Phosphate absorption occurs in jejunum calcitriol, low pH favours their absorption while phytate reduces its absorption. Serum phosphate level is about 3-4 mg/dl and reduced in renal rickets, vitamin D deficient rickets and in diabetes mellitus. Phosphate excreted by kidney in the form of urine. Phosphate is mainly involved in mineralisation of the bone from chondrocytes and osteoblast. The process of mineralisation begins with hydroxyapatite formation from calcium (Ca + 2) and inorganic phosphate. Calcium incorporated through annexin calcium channel here as inorganic phosphate from type III sodium inorganic phosphate transporter and from PHOSPHO1. Hydroxyapatite penetrate the matrix vesicle and elongate due to tissue non-specific alkaline phosphatase (TNAP) and deposit in collagen fibre spaces [35]. The role of phosphorous in bone mineralisation shown in **Figure 2a**. Osteomalacia resulting from hypophosphatemia occurs through fibroblast growth factor signalling (FGF) [36] that links with ageing process [37]. Reduced phosphate levels inside the cell leads to increased FGF 23 levels in the serum and acts by inhibiting calcitriol, PTH, 1α-hydroxylase and stimulating 24-hydroxylase [38]. The signalling pathway connecting phosphorous deficiency and ageing shown in **Figure 3**.

2.2 Potassium (K)

Potassium is principal intracellular cation required daily about 3-4 g that is present majorly in banana, orange, potato, chicken, and liver. It helps regulate fluid balance, nerve signals and muscle contractions and beneficial aspects include reduction in blood pressure, water retention; prevention of kidney stones, osteoporosis, and protection against strokes. It functions to maintain intracellular osmotic

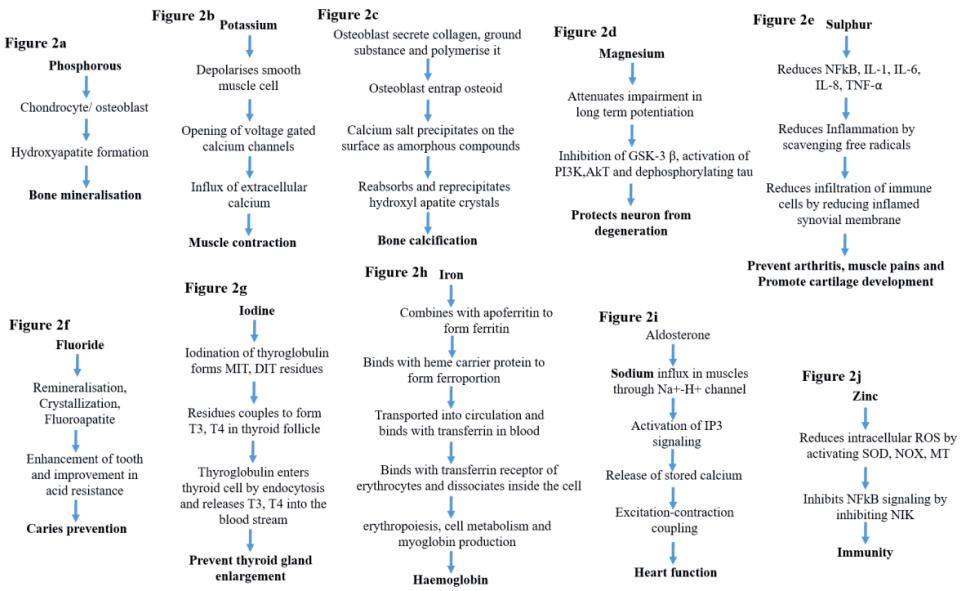

Figure 2.
Role of mineral elements in disease prevention. a: Role of phosphorous in bone mineralisation, b: Potassium involvement in muscle contraction, c: Calcium in bone calcification, d: Magnesium in protection of neuron degeneration, e: Sulphur in prevention of muscle pains and joint pains, f: Fluorine in preventation of dental caries, g: Iodine in thyroid hormones, h: Iron in haemoglobin synthesis, i: Sodium in heart function, j: Zinc in immunity.

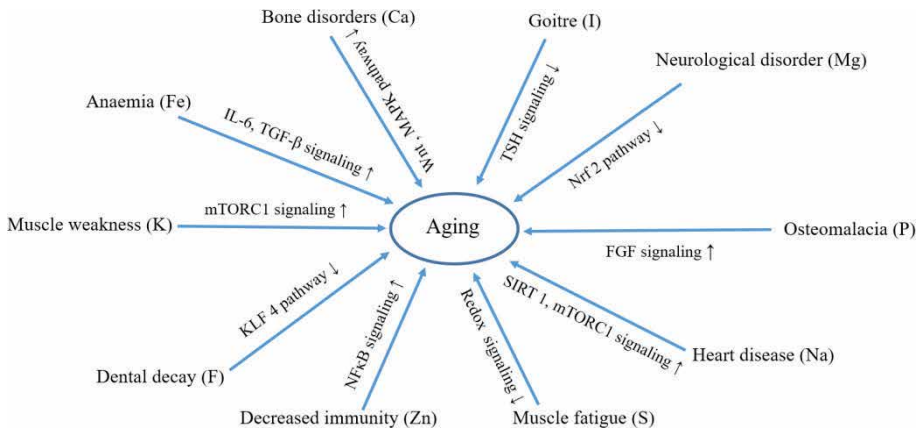

Figure 3.
Deficiency disease leads to aging through disturbed signalling pathway. Mineral deficiencies were shown in parenthesis. Ca: Calcium, I: Iodine, Mg: Magnesium, P: Phosphorous, Na: Sodium, S: Sulphur, Zn: Zinc, F: Fluorine, K: potassium, Fe: Iron. TSH: Thyroid stimulating hormone, Nrf 2-nucleoid erythroid receptor factor 2. FGF-fibroblast growth factor, SIRT1; Sirtuins 1, mTORC1: mammalian target of rapamycin complex 1, NFκB: Natural factor kappa beta, IL-6: interleukin 6, TGF-tumor growth factor, MAPK-mitogen activated protein kinase, Wnt-Wingless-related integration site.

balance, regulation of acid–base balance, required for transmission of nerve impulse, and necessary for biosynthesis of proteins. Plasma levels are 3.4-5 mEq/L absorbed through intestine excreted in form of urine. Deficiency diseases include muscle weakness, mental confusion. Potassium ion present on the cells as potassium ion channels and various types of potassium ion channels include ATP-sensitive K channels (KATP), voltage-dependent K channels (Kv), Ca2+ – and voltage-dependent K channels (BKCa), inward rectifier K channels (Kir), and tandem two-pore K channels (K2P) their activity varies in different types of diseases [39]. Potassium as

known to play a role in Na + -K+ ATPase for effective muscle contraction [40] and motor regulation is by ATP driven potassium channels [41]. ATP driven potassium channel deficiency affected resting tension of skeletal muscle [42] deficiency of potassium ions alters sodium potassium pump of skeletal muscle and augments its contraction in ageing [43]. According to previous reports, high potassium levels depolarizes smooth muscle cells that opens up voltage gated calcium channels resulting in entry of calcium ions inside the cells thereby leading to activation of smooth muscle contraction [44] The role of potassium in muscle contraction shown in **Figure 2b.** It had reported that activation of mTORC1 signalling correlated with decline in muscle mass [45, 46] activated mTORC1induces oxidative stress that leads to protein degradation, autophagy and necrosis showing an aged phenotype [47]. The signalling pathway connecting potassium deficiency and ageing shown in **Figure 3**.

2.3 Calcium (Ca)

Biological availability of calcium is green leafy vegetables, nuts, seafood, cereals etc. Cow's milk is rich source of calcium and required daily about 0.8-1.0 g/day. Calcium plays an important role in development of bones, muscle contraction, blood coagulation, nerve transmission, membrane integrity, activation of enzymes, intracellular messenger, contact inhibition, nerve excitability, skeletal muscle integrity and maintenance, and cardiac tone. Factors promoting calcium absorption include low pH, parathyroid hormone, vitamin D, lactose. Most of blood calcium is in plasma and ranges about 9-11 mg/dl. Factors regulating plasma calcium include calcitriol, parathyroid hormone, and calcitonin. Calcium excreted mostly through intestine and partly by kidneys. Deficiency of calcium leads to hypocalcemia and shown signs such as fragility of bone, muscle cramping, and dry skin. Deficiency diseases include rickets osteomalacia, osteoporosis. Evidences reveal that calcium is involved in bone calcification where osteoblasts secrete collagen as ground substance and polymerises it then osteoblast entrap osteoid and calcium salts precipitates as non-crystalline amorphous substance. Reabsorption and reprecipitation of hydroxyapatite crystals makes bone calcified. Existing reports evidence that stimulation of PGC-1α signalling regulate osteoporosis and ageing [48]. The role of calcium in osteoblast calcification shown in **Figure 2c**. Recent reports reveal that Wnt, MAPK, oestrogen pathways are targets for osteoporosis and ageing, it had shown that Wnt pathway responsible for production of sclerotin is dysregulated and MAPK pathway altered in osteoporosis [49]. The signalling pathway connecting calcium deficiency and ageing shown in **Figure 3**.

2.4 Magnesium (Mg)

Sources of this mineral include milk, meat, fruits, and cereals. Biochemical functions include formation of bone, teeth, neuromuscular irritability, and cofactor for enzymes (kinases). Daily intake is 300-350 mg, serum concentration is 2-3 mg/dl and deficiency leads to convulsions, neuromuscular irritation, uraemia, and rickets. Magnesium absorption occurs in intestine alcohol inhibits it whereas parathormone enhances it. Causes of magnesium deficiency include alcohol abuse, poorly controlled diabetes, excessive or chronic vomiting and/or diarrhoea. Research on neurodegenerative diseases reveal magnesium had neuroprotective role by inhibiting influx of amyloid β from blood and promote its clearance [50] furthermore it attenuates impairment in long-term potentiation and impaired recruitment of synaptic proteins through activation of PI3K/Akt and inhibition of GSK3 β thereby reducing neuronal damage [51]. To date several reports indicate that Nrf-2

an antioxidant responsive protein plays a role in protection of cells from oxidative stress and essential for optimal activity inside the cell [52]. The role of magnesium in neuro degeneration shown in **Figure 2d.** Dysregulated Nrf-2 activity in neuro-degenerative diseases linked to ageing [53, 54]. The signalling pathway connecting magnesium deficiency and ageing shown in **Figure 3**.

2.5 Sulphur (S): Egg white, chicken, fish, beef are major sources of sulphur. Daily intake is 14 mg for healthy adult and distributed in nails, hair, and skin. Sulphur plays a role as antioxidant, anti-inflammation, metal transport, free radical scavenging, protein stabilisation, xenobiotic detoxification, metabolism of lipids. Sulphur resides inside the body in organic form as methionine, cysteine, and cysteine functions as part of vitamins such as thiamine, biotin, and coenzyme A and excreted through oxidised form as taurine and cholic acid. Deficiency diseases are almost unknown. Although reports revealed that, sulphur containing amino acids in the form of methionine and cysteine forms creatinine, carnitine and coenzyme. Sulphur in the form of methylsulfonylmethane (MSM) acts to prevent muscle pains and joint pains through reduction of pro-inflammatory cytokines (NFkB, IL-1, IL-6, IL-8, TNF-α) [55–57] and decreased infiltration of immune cells by reducing inflamed synovial membrane [58, 59]. The role of sulphur in muscle pains and joint pains shown in **Figure 2e**. An essential for muscle functioning and deficiency leads to muscle impairment and aged phenotype. Aged muscle has altered Redox signalling [60–62] and exercised individuals in their lifetime had preserved enough muscle fitness comparable to younger ones [63] whereas NAD^+ treatment [28] reverse these effects. Strenuous exercise result in muscle damage [64] and dysregulated redox response within the muscle increase in transient ROS/RNS. This clearly explains redox mechanisms operate with ageing and contraction of skeletal muscle can activate a number of transcription factors thereby affecting gene expression of specific cellular pathways. The signalling pathway connecting sulphur deficiency and ageing shown in **Figure 3**.

3. Deficiency of minor mineral elements and lifespan

3.1 Boron (B)

It occurs mostly in soil and water; dietary sources include leafy vegetables, pineapple, dry fruits, lemon, nuts, and berries and daily intake is <20 mg. It is ingested through diet and found higher quantities in hair, nails, bone whereas fat tissue being low [65]. It is absorbed into the intestine through boric acid and stored in tissues. The toxic effects of boron include DNA damage and repair and has effect on protein folding and stability. In infants, excess of boron leads to anaemia, seizures, erythema, dermatitis, cardiac problems [66–68]. Chronic exposure leads to disorders of brain, kidney, and testis (88). Boron determination utilises spectrophotometry [69], spectrofluorimetry [70], potentiometry [71], inductive coupled plasma atomic emission spectroscopy [72], and inductive coupled plasma mass spectrometry techniques [73]. Beneficial effects include reduction in sterility, osteoporosis, inflammation, coagulation, and cancer. Its application widely relays on food and medicinal sector.

3.2 Fluoride (F^-)

Fluoride levels abundantly found in barley, rice, cassava, canned fruits and least in food grain, breast milk, beverages and daily intake is about 2 ppm. Fluoride levels in the environment is taken up either by food, water or inhaled by air, drugs and

reach the digestive tract for metabolism and distributed inside the body bone, soft tissue, milk, tooth. The factors that influence the fluoride metabolism inside the body include acid–base disorders, hormones, physical activity, cardiac rhythm, and diet. Fluorine functions as prevention of dental caries, necessary for development of bones. The mechanism of action of fluoride inside the body involves inhibition of demineralisation of enamel. A small amount may substantially contribute to health benefits that include dental caries, decreases acid production. High levels leads to alterations in cell architecture, abnormalities in hepatic and renal systems. Fluoride poisoning inside the cells diagnosed by contraction of muscle, stiffness of body, failure of respiratory and cardiac systems. The methods for removing excess of fluorine done using coagulation-precipitation, electro coagulation, adsorption etc. Excreted through faeces, urine. Deficiency diseases include dental caries, osteoporosis. Fluoride helps in remineralisation, crystallisation and Fluoroapatite formation through enhancement of tooth and improves against acid resistance thereby preventing dental caries [74]. The role of fluorine in dental caries shown in **Figure 2f**. Reports reveal that klotho/KLF4 protein is involved in secretion of saliva from salivary gland and attenuation of KLF4 pathway thereby inactivating mTOR, AMPK, cyclin D1 that leads to dental caries [75]. The signalling pathway connecting fluoride deficiency and ageing shown in **Figure 3**.

3.3 Iodide (I^-)

It is abundant in seafood, iodised salt and daily intake is about 150-200 ug. It is component of thyroid hormones stored in the form of thyroglobulin and toxicity symptoms include thyrotoxicosis, goitre. Iodine is mainly absorbed through small intestine but also occurs through skin and lungs. Plasma level is 4-10 mg/dl. Iodine mainly excreted through kidney but also through skin, milk saliva and bile. Deficiency causes cretinism, goitre, and myxoedema. It is evident from existing reports that iodine uptake by thyroid cells occurs with the help of sodium iodine symporter and translocates to apical membrane fuses with thyroglobulin with the help of thyroperoxidase to form monoiododthyronine (MIT), diiodothyronine (DIT) in thyroid follicle cells. Coupling of MIT & DIT results in triiodothyronine (T3) & tetra iodothyronine (T4) which is internalised through endocytosis that releases free T3, T4 into the blood stream. Iodine deficiency leads to uptake of more thyroid-stimulating hormone (TSH) into thyroid cells for production of thyroid hormones (T3 & T4) which results in enlargement of thyroid gland to form goitre [76]. Age associated abnormality of thyroid gland is not consequence of ageing but result of thyroid autoantibodies that leads to age associated diseases [77]. The role of iodine in goitre shown in **Figure 2g.** Disturbed TSH signalling found in ageing individuals due to reduced release of TRH and less production of TSH thereby lowering the thyroid gland response to TSH with concomitant release of T3 and T4 [78] and enhances Ras activity that leads to increase of thyroid gland cell proliferation [79]. The signalling pathway connecting iodine deficiency and ageing shown in **Figure 3**.

3.4 Iron (Fe)

Iron (non-heme) abundantly found in cereals, pulses, fruits, vegetables whereas heme is from poultry, fish and daily requirement is about 10-15 mg. Iron present in the form of heme transports oxygen, involved in electron transport chain, required for phagocytosis in form of peroxidase. Iron is absorbed in stomach and duodenum low pH, vitamin C enhances its absorption whereas phytate and oxalate interfere its absorption. Enterocytes absorb iron through metal transporter 1 protein and

gets metabolised (heme) through heme oxygenase-1 [80, 81]. Inhibitors of iron absorption includes phytic acid [82], polyphenols [83], and calcium [84] whereas ascorbic acid is enhancer [85]. Iron is transported inside the body through circulating proteins namely transferrin, lactoferrin, ferritin, heme proteins [86]. Iron regulation inside the cells occurs by 2 mechanisms one is by binding of iron responsive elements (IRE) [87] to iron responsive proteins (IRP) and other by Hepcidin. Gene mutations of transferrin receptor 2, haemochromatosis, haemochromatosis type 2, hepcidin antimicrobial peptide (HAMP) [88] for impaired expression had observed. Iron storage inside the body is by ferritin [89] in liver, spleen, bone marrow [90]. Bodily iron is mostly excreted in form of blood through menstrual release and other forms includes skin and gastro intestinal tract [91] but not through urine. Iron deficiency results in depletion of iron and primary cause is low bioavailability of iron. It also occurs through pregnancy, menstruation, and pathologic conditions [92, 93]. Anaemia is the sign of iron deficiency [94]. Iron deficiency overcome by improvement in iron uptake and bioavailability, supplementation of iron with food and its fortification. Deficiency diseases include hypochromic microcytic anaemia. Reports evidence that iron ($Fe^{+}2$) is absorbed by duodenal cells and binds with apoferritin to form ferritin which then binds to heme carrier protein (HCP) to form ferroportion (FPN). Ferroprotein is either stored in liver or transported in the blood, combines with transferrin in blood and reach erythrocytes that then binds to transferrin receptor and internalised into the cell and gets dissociated with the help of divalent metal carrier transporter 1 and performs functions such as erythropoiesis, cell metabolism, myoglobin production in muscles. Heme combines with myoglobin to form haemoglobin [59]. Recent reports reveal that PR domain zinc finger protein 8 (PRDM8) gene had a role in premature ageing of haematopoietic cells through DNA methylation that leads from aplastic anaemia (AA) patients independent of telomere attrition a haemoglobin disorder [95]. The role of iron in haemoglobin synthesis shown in **Figure 2h.** Reports also state that anaemia resulting from erythropoiesis of haematopoietic ageing of intrinsic altered microenvironment had upregulated IL-6, TGF-β signalling [96]. The signalling pathway connecting iron deficiency and ageing shown in **Figure 3**.

3.5 Molybdenum (Mo)

The daily intake of molybdenum was 75-250 ug and toxicity characterised by gout and joint pains. Molybdenum is present as cofactor for nitrate reductase, Xanthine oxidase and sulphite oxidase enzymes. Molybdenum cofactor biosynthesis occurs in steps formation of precursor Z from GTP, synthesis of molybbdeoprotein from precursor Z, addition of adenyl group to molybdoprotein and its insertion [97]. Molybdenum uptake inside the cells occurs with the help of ATP binding cassette transporters [98]. Molybdenum deficiency results in improper functioning of enzymes responsible for specific metabolic pathways in which they were involved and leads to metabolic diseases such as Xanthinuria, Hyperuricemia, and neurodegeneration. Deficiency diseases are almost unknown but some reports reveal its deficiency leads to chrons disease.

3.6 Sodium (Na)

Abundantly found in common salt and other sources include leafy vegetables, milk, eggs, and nuts and daily intake is about 5-10 g. Absorbed as sodium ions and circulates inside the body in plasma and plasma levels were 135-145 mEq/L. It is cheif extra cellular cation regulates acid–base balance and involved in osmotic pressure. It is involved in activation & transmission of nerve impulse, absorption of biomolecules

and aldosterone. High levels were observed in cushions disease and low levels were observed in addisons disease. Excreted from kidney in the form of sodium chloride through urine or as phosphate and other routes is by sweat. Deficiency diseases are almost unknown but reports reveal that higher risk of cardiovascular disease with low sodium intake [99]. Sodium inside the cells were present as sodium channels as (sodium-potassium ATPase, sodium-proton antiporter) the role of sodium in heart function is mostly presented by stimuation of aldosterone which enhaces its influx into the cell and activates inositol 1,4,5 tri phosphate (IP3) [100, 101]. Activated IP3 releases stored calcium from endoplasmic reticulum and makes excitation coupled to contraction for effective heart function [102]. The role of sodium in heart function shown in **Figure 2i**. SIRT1, mTORC1 regulate cell balance between cell growth and survival. Activation of SIRT1 along with PGC-1α, AMPK and inhibition of mTORC1 along with Akt act to prolong cell longevity and retard cardiac ageing. Autophagy underlies the activation of SIRT1/PGC-1 α/AMPK and inhibition of Akt/mTORC1 responsible for cardiac ageing. Chronic heart failure involves deficient autophagy phenomenon through hyperactivation of Akt/mTORC1 and suppression of SIRT1/PGC-1 α/AMPK pathway that finally leads to cardiac ageing [103, 104]. The signalling pathway connecting sodium deficiency and ageing shown in **Figure 3**.

3.7 Zinc (Zn)

Zinc mostly found in meat, cabbage, dates, mushrooms etc. and daily intake is 10-15 mg. Exposure of zinc is mainly by three ways inhalation, dermal exposure, oral exposure [105] and excess zinc shows symptoms such as abdominal pain, nausea, anaemia, gastrointestinal effects. Zinc plays an essential role as structural, catalytic, mild deficiency causes oligospermia, hyperammonemia [106]. Zinc is absorbed in duodenum phytate inhibits absorption whereas amino acids enhances its absorption. Oral uptake of zinc absorbs through small intestine and distributed in serum by binding to albumin, α-microglobulin, and transferrin [107]. Zinc homeostasis occurs mainly with the help of transport proteins namely Zinc importer (ZIP) and zinc transporter (ZnT) [108] which then binds to metallothionin, and sequester to other cell organelle. Beneficial aspects of zinc were antioxidant [109], antidepressant, antidiabetic [110], delayed wound healing, and anticancer [111]. Toxic effects of zinc observed when it crosses more than 100-300 mg/day typical symptoms include reduction of HDL and cholesterol levels, vomiting, lethargy, and fatigue. Serum zinc levels is about 100 mg/dl. Excretion of zinc occurs mainly by kidney, skin, and intestine. The role of zinc as immune protector well studied as anti-inflammatory and performs its action through reducing intracellular ROS by activating superoxide dismutase (SOD), NADPH oxidoreductase (NOX), metallothionin (MT) thereby suppressing inflammatory pathway (NFkB) and reduces it [112]. The role of zinc in immunity shown in **Figure 2j**. Zinc deficiency induces oxidative stress activates transcription factors NFkB, AP 1 through NFkB signalling in ageing process [113, 114]. The signalling pathway connecting zinc deficiency and ageing shown in **Figure 3**.

4. Conclusion

Minerals play an important role in daily life ranging from nuts to leafy vegetables. Minerals mainly function as cofactors along with enzymes to show their metabolic effect. Minerals form holoenzymes in metabolism of biomolecules and help in cellular vital process for cell survival. In their absence, the show some deficient metabolic effects and required in small amounts to function effectively. Intake

Mineral	Physiological function	Mechanism of action	Deficiency disease	Signalling pathway associated with ageing
Phosphorous (P)	Formation of high energy phosphates, nucleic acids, nucleotide coenzymes	Bone mineralisation through hydroxyapatite formation [35]	Osteomalacia	FGF signalling [36, 37]
Potassium (K)	Chief cation of intracellular fluid, osmotic balance, muscle function	Contraction of smooth muscle cell [44]	Muscle weakness, mental retardation	mTORC1 signalling [47]
Calcium (Ca)	Development of bones, muscle contraction, blood coagulation, nerve transmission, intracellular messenger etc.	Bone calcification through formation of hydroxyl apatite crystals	Rickets, Osteoporosis, Osteopetrosis (marble bone disease)	Wnt, MAPK pathway [49]
Magnesium (Mg)	Constituent of bones, cofactor for kinases	Protects neuronal cell death by activating PI3K/Akt signalling [51]	Neuromuscular weakness, muscle irritation	Nrf 2 pathway [53, 54]
Sulphur (S)	Constituent of vitamins, heparin, chondroitin sulphate	Reduces muscle pain and body pain [55–57]	Muscle fatigue, convulsions	Redox signalling [60–62]
Fluorine (F)	Formation of bones and teeth	Prevents dental caries by remineralisation of enamel and improving acid resistance [74]	Dental caries	KLF 4 pathway [75]
Iodine (I)	Constituent of thyroxine, triiodothyronine	Prevents thyroid enlargement through T3 &T4 [76]	Goitre, Myxoedema	TSH signalling [78]
Iron (Fe)	Transports oxygen in constituent of heme	Haemoglobin formation through erythropoiesis [59]	Hypochromic microcytic anaemia	TGF-β signalling [96]
Sodium (Na)	Chief cation of extracellular fluid, osmotic balance, acid–base balance, nerve function	Regulates heart function through IP3signaling by aldosterone [100–102]	Heart disease	SIRT1, mTORC1 signalling [103, 104]
Zinc (Zn)	Cofactor for alcohol dehydrogenase, carbonic anhydrase, lactate dehydrogenase	Reduces intracellular ROS by activating SOD, NOX, MT [112]	Growth retardation, hypogonadism, decreased immunity	NFkB signalling [113, 114]

Abbreviations: FGF-fibroblast growth factor, SOD-superoxide dismutase, NOX-NADPH oxidase, MT-metallothionin, T3-tri iodothyronine, T4-tetra iodothyronine, PI3K-Phosphatidyl inositol 3 kinase, MAPK-mitogen activated protein kinase, Wnt-Wingless-related integration site, Nrf 2-nucleoid erythroid receptor factor 2, TSH-thyroid stimulating hormone, TGF-tumour growth factor, SIRT 1-sirutin1, mTORC1-mammalian target of rapamycin complex 1, NFkB-natural factor kappa beta.

Table 1.
Summary of mineral elements mechanism of action and association with longevity.

varies from infants to adults, gender excess amounts shows hyper forms, and low amounts leads to hypo effects. Mineral deficiencies mostly show aged phenotype and age related diseases have mineral deficiencies. In their absence cell, survival pathways are mostly non-functional and leads to decreased metabolic function that is characterised by aged phenotype. Minerals classified mostly upon their requirement as major (phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S)), minor/trace/rare (Boron (B), chlorine (Cl), chromium (Cr), fluoride (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), sodium (Na), vanadium (V) and zinc (Zn)). A selected mineral with their function importance in mammals have been described in detail in which Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulphur (S), Fluoride (F^-), Iodide (I^-), Iron (Fe), Sodium (Na), Zinc (Zn) along with mechanism of action and its diseased mechanism associated with ageing. Phosphorous is involved in bone mineralisation from osteocyte through hydroxyl apatite formation and deficiency leads to osteomalacia that related to ageing through increased fibroblast growth factor signalling. Potassium is involved in muscle contraction and its deficiency leads to muscle weakness and shows aged phenotype through enhanced mTORC1 signalling. Calcium is involved in bone calcification through hydroxyl apatite crystals its deficiency leads to bone disorders shows aged phenotype through dysregulated Wnt, MAPK pathway. Magnesium is involved in protection of neuron from degeneration through inhibition of GSK3β signalling and hyper activation of PI3K, Akt signalling and shows aged phenotype through dysregulated Nrf 2 pathway. Sulphur is involved in prevention of muscle pains and joint pains by reducing inflammation by scavenging free radicals its deficiency leads to muscle fatigue shows aged phenotype through reduced redox signalling. Fluorine is involved protection of enamel layer by remineralisation, crystallisation of dentine and enhancement in acid resistance its deficiency leads to dental caries which is also an aged phenotype due to disturbed KLF4pathway. Iodine is necessary for thyroid gland for production of thyroid hormones deficiency of it leads to goitre that is characterised by thyroid gland enlargement seen mostly in aged people or people taking iodine deficient diets that occurs through reduced TSH signalling. Iron is necessary for body for haemoglobin synthesis for oxygen transport and its deficiency leads to anaemia an aged phenotype occurs through enhancement in IL-6, TGFβ signalling. Sodium shows its effect by action of aldosterone on muscle cells and helps in heart function deficiency leads to heart diseases an aged phenotype occurs through increased SIRT1, mTORC1 signalling. Zinc well known for immune defence through inhibition of NFκB signalling deficiency leads to reduced immunity through enhancement of this signalling. A summary of different minerals and their mechanism of action along with their associated signalling pathway with ageing had described in **Table 1**.

Appendices

Ca	calcium
I	iodine
Mg	magnesium
P	phosphorous
Na	sodium
S	sulphur
Zn	zinc
F	Fluorine
K	potassium
Fe	iron

TSH	thyroid stimulating hormone
Nrf	nucleoid erythroid receptor factor
FGF	fibroblast growth factor
SIRT	sirtuins
mTORC	mammalian target of rapamycin complex
NFkB	natural factor kappa beta
IL	interleukin
TGF	tumour growth factor
MAPK	mitogen activated protein kinase
Wnt	Wingless-related integration site
FGF	fibroblast growth factor
SOD	superoxide dismutase
NOX	NADPH oxidase
MT	metallothionin
T3	tri iodothyronine
T4	tetra iodothyronine
PI3K	phosphatidyl inositol 3 kinase
ZIP	zinc importer
ZnT	zinc transporter
GSK3β	glycogen synthase kinase 3β
ROS	reactive oxygen species
RNS	reactive nitrogen species
HDL	high density lipoprotein
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator *1-alpha*
IP3	inositol 1,4,5 tri phosphate
ATPase	adenosine tri phosphatase
GTP	guanosine triphosphate
PRDM8	PR domain zinc finger protein 8
AA	aplastic anaemia
HCP	heme carrier protein
FPN	ferroportion
IRP	iron responsive proteins
IRE	iron responsive elements
HAMP	hepcidin antimicrobial peptide
MIT	monoiododthyronine
DIT	diiodothyronine
TSH	thyroid-stimulating hormone
AMPK	adenosine monophosphate kinase
DNA	deoxyribose nucleic acid
RNA	ribose nucleic acid
NAD	nicotinamide adenosine dinucleotide
TNF	tumour necrosis factor
MSM	methylsulfonylmethane
FGF	fibroblast growth factor signalling
PTH	parathormone
TNAP	tissue non-specific alkaline phosphatase
ULK 1	Unc-51 like autophagy activating kinase (ULK1/2)
MMP	matrix metallo proteinase
ICAM	inter cellular adhesion molecule
iNOS	induced nitric oxide synthase
COX	cyclooxygenase
RDA	recommended dietary allowance

Author details

Nyshadham S.N. Chaitanya[1*] and Sibani Sahu[2]

1 Department of Animal Biology, School of Life Sciences, University of Hyderabad, TS, India

2 Department of Human Genetics, Andhra University, Visakhapatnam, AP, India

*Address all correspondence to: nsnchaitanya8@uohyd.ac.in

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Chapter 4

Organoleptic, Sensory and Biochemical Traits of Arabica Coffee and Their Arabusta Hybrids

Kahiu Ngugi, Jane Jerono Cheserek and Chrispine Ogutu Omondi

Abstract

Coffee as a cash crop, reduces food insecurity by providing regular incomes and is a major foreign exchange earner in more than fifty tropical countries where it is grown either as Arabica (*Coffea arabica*) or Robust (*Coffea canepora*). In Kenya which grow some Robusta but mostly Arabica coffee, the production has been declining, mainly because world coffee prices have plummeted to about 5 USD for a 650Kg of un-hulled beans per acre. The only way world prices are likely to increase and benefit the small-scale farmers, is by improving the cup quality and enabling these countries to sell their coffee in specialty markets. This review, underscores the importance of analyzing and estimating organoleptic, sensory and biochemical compounds diversity in Arabica coffee, since these are the factors that determine cup quality. In an attempt to do so, the chapter presents experimental data that analyzed various sensory and organoleptic traits of Arabica coffee and their Arabusta hybrids that proves that tremendous genetic diversity exists in coffee genotypes grown in Kenya and it is possible to utilize this genetic variation to improve cup quality.

Keywords: Arabica coffee, Arabusta hybrids, biochemical traits, cup quality, genetic diversity

1. Introduction

1.1 Arabica coffee production in Kenya

Coffee is an export oriented crop that contributes significantly to the economic growth of 80 developing countries of the world. Approximately, 125 million people in 50 developing countries of Africa, Latin America and Asia produce and sell coffee as their main source of income [1]. *Coffea canephora* Pierre ex A. Froehner or Robusta coffee, makes 30% of the world's commercial coffee and 80% of the production in Africa, with Uganda being among one of the top most producers [2]. Globally, coffee fetches more than $ 79 billion US dollars in the world markets [3] and in Ethiopia, when the genetic value of resistance to diseases, pests, high yield and low caffeine is considered, that value rises to between US $420 - $1.45 billion [2]. Arabica coffee constitutes 70% whereas Robusta, contributes 30% of the total world product and export [2].

Arabica coffee or *Coffea arabica*, earns Kenya, US$230 million, and is the most important export commodity after horticulture, tourism and tea. Kenya has some

of the best quality Arabica coffees globally, but the production has declined from 130,000 metric tons in 1988 to about 45 metric tons, at present [4].

Being a tropical crop, *Coffea* requires specific environmental conditions for commercial cultivation. The quality of developing bean from flowering to ripening is influenced by altitude, latitude, temperature, rainfall, soil, sunlight, wind and humidity [5–7]. Arabica coffee grows at altitude ranges of 1200–1800 meters above sea level (masl) rainfall amounts range between 400 and 600 mm per season though it is also cultivated at 400 m above sea level as long as there is no frost. Robusta coffee cultivation on the other hand is mostly grown at lower altitudes, between sea-level till to an altitude that limits its vegetative growth.

Compared to Arabica, Robusta coffee has smaller bean sizes, poor flavour and high bitterness content but is tolerant to coffee leaf rust disease, has resistance to white stem borer but is susceptible to drought stress [8, 9]. Due its poorer quality characteristics, Robusta coffee global market value is lower than that of *C. Arabica*.

1.2 Coffee origin and diversity

The genus *Coffea* L has over 105 species, prevalently found in Africa and Madagascar [9, 10]. *Coff*ea belongs to the genus of the Rubiaceae family, is indigenous to Africa and is widely distributed in the tropics [11]. The centres of origin of Arabica coffee are the mountainous rain forests of Ethiopia, the western and eastern slopes of the Great Rift Valley and the Boma plateau of the Sudan. Wild species of *C. canephor* are found in Guinea, Uganda, Sudan, Northern Cameroon Southern Angola and in the Congo forests [11–13]. *Coffea liberica* Bull. Ex. Hiern, known for its resistance to diseases, insect pests, adaption to low elevation, is native to the tropical forests of Liberia and Cote de Ivoire [14] whereas *Coffea mascara* characterized by low levels absence of caffeine is found in the forests of Madagascar Mauritius and Reunion [11–13].

The diverse existence of the genus *Coffea* in Uganda, with species such as *C. eugenioides* S. Moore, *C. excelsa* Chev and *C. spathicalyx* K. Schum., suggests that the country is the centre of origin [11, 13]. Whereas three of the genus *Coffea* species are economically important, coffee production and its industry depend on two species only; Arabica and Robusta coffee [15, 16]. The third important species of coffee, *Coffea liberica* is produced mainly in Liberia, Java, Malaysia and the Philippines but because of its low yield and poorer quality, it is used only for local consumption. With advanced breeding techniques, commercial interspecific hybrids such as Arabusta (*C. arabica* x *C. canephora*) have been developed. Blending coffees from the two species at varying ratios probably produces the preferred consumer flavours at lower costs [17]. With the exception of *C. arabica* that is tetraploid and self-fertile (2n = 4x = 44), all the other species in the genus *Coffea* are diploid (2n = 2x = 22), with gametophytic self incompatibility and therefore there exists gene flow between them and the cultivated *C. canephora* [18]. Given its allopolyploidy and self-pollinating nature, *C. arabica* is characterized by low genetic diversity leading to a narrow genetic base [1].

1.3 Objectives in coffee improvement programs

Since the quality of coffee is the key determinant of prices in the world markets, genetic improvement of Robusta coffee organoleptic cup characteristics, yield and caffeine is being undertaken by many researchers throughout the world in an attempt to match Arabica coffee characteristics in order to stabilize and sustain development in the coffee growing areas [17]. Promoting coffee liquor quality would add value, enhance income and increase the competitiveness of the world coffee prices. To sustain value, most coffee improvement programs are aiming to select and breed for cultivars with genetically superior organoleptic cup quality and

are using modern, molecular marker tools such as SSRs, SNPS in combination with, physiological and biochemical green bean tools [17]. In the same manner, the influence of environmental factors such as soil texture, nutrient element composition, altitude, rainfall, temperature that directly or indirectly contribute to coffee quality is given priority and is determined alongside genetic traits [19, 20].

2. Factors that influence coffee quality

Coffee quality is influenced by factors such as the genetics, handling procedures, ecological conditions and agricultural practices. According to the International Organization for Standardization (ISO) quality is "the ability of a set of inherent characteristics of a product, system or process to fulfill requirement of customers and other interested parties" [21]. Depending on the actors in the value chain coffee quality could refer to, the variety, price of coffee, the consuming culture, tonnage or on bean physical characters and biochemical compounds in the green bean. It is the effect of cup quality that determine commercial coffee grade and not the bean size.

2.1 Organoleptic cup quality

Coffee bean physical appearance is an integral indicator of cup quality, but it is the assessment by consumers through their human sensory organs and consumption habits that determine the final quality [17]. The most important attributes are; fragrance, aroma, flavour, bitterness, sweetness, saltness, acidity, mouth feel, aftertaste and cup balance. Fragrance originates from the smell of roasted or ground beans whereas aroma emanates during brewing with boiled water. Aroma helps evaluate flavour and coffee liquor brightness [22]. Flavour is described as an individual person feeling of appreciation during the tasting of the coffee brew taste, which does also include aroma. Fat stabilizes flavour compounds formed during roasting [18, 22, 23]. The undesirable coffee bitter taste in the mouth is positively correlated with the total dissolved coffee solids. High levels of saltiness and undesirable aroma are associated with high levels of potassium in Robusta coffee. Coffee brew taste is less preferred by consumers when potassium and caffeine are at lower levels [24]. Coffee medium roast has less soluble solids, a higher acid content, and more stringent aroma compared to the dark roast [25]. Roasted beans that are less bitter but have a high sweet taste is rated high by many consumers.

Acidity is regarded as the sharp and pleasing sweet to fruity/citrus taste close to the dry taste experienced on the back sides of the tongue while drinking red wine. Perceived acidity in coffee does not necessarily correlate with coffee pH, but is a result of the acids such as aliphatic, chlorogenic, alicyclic carboxylic and phenolic acids that are developed during medium and dark roasted stages. Cup acidity is influenced by high concentrations of citric acid, malic acid, and acetic acid and low concentrations of phosphorus and potassium. Acidity is thought to be influenced by phosphoric acid levels,though it may not directly correlate with perceived acidity [26]. Mouth-feel or liquor body is determined by micro fine fiber and fat content. Liquor weight is caused by micro fine fiber particles whereas texture is derived from oils extracted from ground coffee suspended in the brew. Brew colloids are formed when oils coagulate around fibers suspended in the brew. Coffee weight and texture (slipperiness) in the tongue is compared to pure water and is determined by the micro fine fiber and fat content [27]. Viscosity is caused by proteins and fibers in the brew and is normally denser in medium roasted and dark coffees than in lighter roasted beans (http://www.coffeeresearch.org/science/news.htm) [24].

Taste is normally perceived as the feeling in the mouth after sipping the beverage whereas aftertaste is perceived as the lingering remnant sensation experienced at the back of the throat after swallowing but often changes over time [24]. In a balanced cup, a complementary synergistic combination of flavor, aftertaste, mouth feel and bitter/sweet aspect ratio occurs when the four attributes are in equal intensities [24].

Soft, pleasing and delicate taste derived from acidity and sweet coffee is obtained from fruit acids, high sugars levels and chlorogenic acids (http://www.ico.org/vocab.asp) [28].

There are four major reactions that determine to a great extent of the aroma of roasted beans. Firstly is the Maillard reaction that occurs between nitrogen containing substances such as amino acids, proteins, trigonelline and serotonine with carbohydrates such as sugars. Degradation of individual amino acids, particularly sulphur amino acids, hydroxy-amino acids and praline is the second reaction. Thirdly, sucrose degrades to aliphatic acids compounds and caramel- like substances that contribute to flavour either as volatile aroma compounds, or non-volatile taste compounds [29–31]. The fourth reaction is the degradation of phenolic acids especially the quinic acid moiety.

Roast bean fat has been shown to be positively significantly correlated with aroma, body, acidity, flavor, aromatic intensity and quality, overall judgment and preference [18, 19, 23, 32, 33]. Higher bean yields produced under favourable environmental conditions have reduced acidity. Caffeine content has been found to be negatively, significantly correlated with cup quality attributes although, [34, 35] reported positive correlation coefficients between preference and acidity and aroma in Robusta coffee hybrids and in commercial clones.

Specialty coffee markets demand distinctive cup attributes such as homogeneity, regularity and reliability. Organoleptic cup attributes have to be stable, for the roaster and the consumer [17]. Evaluation of organoleptic cup attributes and other quality parameters using various scientific methods reveal varietal differences and similarities in genetic traits. Genotypic as well as environmental effects influence cup quality that is determined further by the way cherries and beans are picked, shipped and roasted [36]. Varying cup differences that result from genotypic differences contribute greatly to market value, as is the case for Central America consumers who prefer traditional cultivars (Bourbon, Caturra, Catuai, Pacamara) to newer cultivars derived from the 'Hybrid of Timor' hybridization. In Uganda, where *C. canephora* has evolved over years and traditionally cultivated as a culture, farmers and buyers have been less inclined to consume products of Arabusta hybrids selected on quality and other desirable agronomic traits even when they have resistance to the coffee wilt disease. Genotypes show different cup qualities under different environments. For instance, Blue Mountain genotype, has superior liquor quality when grown under Latin American farmer conditions than when grown by East African farmers [17]. Coffee from Africa tend to have high acidity, low body, sweet fruits, floral and dry wine taste [37].

Coffee from Asian countries such as India, Java, Sumatra, Sulawesi and Papua New Guinea is perceived to have low acidity, high body and smoothness, earthy and spice flavor characteristics [38] whereas Latin America countries such as Brazil, Columbia, Costa Rica, Guatemala, Nicaragua, Mexico, El Salvador, Peru, Panama and Honduras produce coffee with medium acidity and body, intense aroma but has a full spectrum of tastes.

2.2 Biochemical compounds of coffee

The interaction of caffeine, oil, sucrose, chlorogenic acids, and trigonelline is what determines the final cup quality of coffee [39]. Organoleptic factors such as

aroma and taste within the coffee to the biochemical composition of the bean that affects the final cup quality. These biochemical compounds act as aroma precursors and the interaction between then is key to the coffee quality of specific cultivars.

2.2.1 Caffeine

Caffeine (1, 3, 7-trimethyl xanthine), is the main alkaloid found in its natural form in leaves, seeds or fruits in 63 different plant species [40]. This chemical occurs in natural form in leaves, seeds, or fruits of 63 different plant species [40]. The biological role of caffeine in plants has not been clear, although it has been suggested that caffeine protects the plant from pests and that t has an allelopathic effect on seeds affecting their germination [41]. Caffeine is an odorless, white powder with a molecular weight of 194.19 g, melting point of 236°C, sublimation point of 178°C with pH values ranging from 6 to 9 [40].

Robusta coffee has a higher content of caffeine than that of Arabica, with an average value of 2.2%, whereas Arabica has about 1.2% with a range of 0.6 to 1.9% [42, 43]. Liberica has the lowest caffeine content of 1.35% of caffeine whereas Arabusta hybrids follow closely at about 1.72% [44]. Genetic and environmental factors are the major causes of variations of caffeine content in the coffee beans. Different levels of caffeine content in the coffee bean cause various physiological and psychological effects in humans [45–47]. About 80% of administered caffeine (1,3,7-trimethylxanthine) is metabolized by demethylation to paraxanthine (1,7- dimethylxanthine) via liver *cytochrome* P-450 1A2, and about 16% is converted to theobromine and theophylline, (3,7- and 1,3-dimethylxanthine, respectively) [47]. Higher levels of caffeine consumption have been associated with improved performance in human reaction time, verbal memory, and visuospatial reasoning but may also cause heart disease, kidney malfunction, and asthma among other disorders [48].

2.2.2 Carbohydrates

Arabica coffee is more preferred by most consumers than Robusta because it is less bitterness and has good flavour [49, 50]. These characteristics are contributed by the carbohydrates that account for more than 50% of the coffee bean dry weight [8]. During roasting, sucrose is degraded to form the anhydro-sugars and glyypxal that determine flavour and aroma [29]. These compounds react with amino acids through the Maillard reaction to form aliphatic acids, hydroxymethylfurfural, pyrazine and other furans. Furan derivatives are the principal products of decomposition of monosaccharides and higher sugars [51]. The composite roasting is regarded as essential in contributing to the final coffee flavour either being volatile or non-volatile [52]. Sucrose levels in Arabica coffee range from 5.1% to 9.4% in the dry matter of coffee beans which is higher than that of Robusta that range between 4–7% [53, 54].

2.2.3 Trigonelline

Trigonelline, a nitrogenous compound is derived from the methylation of the nitrogen atom of nicotinic acid (niacin) and an alkaloid that has a chemical formula, of $C_7H_7NO_2$ and molecular weight of 137.138 g/mol [55]. Trigonelline is a major source in discriminating between Arabica and Robusta coffees during roasting [56]. Arabica has trigonelline levels ranging from 0.88% to 1.77% dmb whereas *C. canephora* species levels range from 0.75% to 1.24% dmb [53]. Trigonelline is a vitamin B6 derivative with 100% solubility in water and contributes to bitterness

in coffee [54]. Degradation of trigonelline during roasting results in niacin, nicotinamide and a wide range of aroma volatiles, that include pyridines and pyrroles which in turn influence flavour [6, 53].

2.2.4 Chlorogenic acids

Chlorogenic acids (CGA) are the highest occurring polyphenols in coffee and form a significant part of coffee antioxidants [57, 58]. CGA belongs to hydroxycinnamic acids classes that comprise caffeic acid (3,4-hydroxycinnamic acid), ferulic acid (3-methoxy-4-hydroxycinnamic acid), p-coumaric (4-hydroxycinnamic acid), and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) [59]. CGA varies from 4% to 8.4% in Arabica coffee and between 7% to 14.4% in Robusta coffee whereas Arabusta hybrids have intermediate levels [60]. Maillard and Strecker's reaction cause chlorogenic acids to form pigments that affect taste and flavour [61].

2.2.5 Lipids/oils

Oil which is produced during roasting process, is the key determining factor of flavour and its quantity in the green bean is cultivar specific. The most important lipids in Arabica beans are the fatty acids that include the triacylglycerols, sterols, and tocopherols which are also found in vegetables [62]. Arabica coffee contains about 15% lipids compared to 10% in, Robusta coffee. Most lipids in the green coffee bean are located in the endosperm whereas the rest is found on the outer layer of the beans [63].

3. Organoleptic attributes of arabusta hybrids from experimental data

3.1 Materials and methods

3.1.1 Experimental materials and site

Field trials were conducted in Siaya and Busia counties of Kenya, in 2018/2019. Nineteen genotypes including seven Arabusta hybrids, six different backcross derivatives of Arabica to Arabusta hybrids, Congusta, Congensis, Arabusta cultivar, Robusta, *C arabica* (Batian) and *C arabica* (Ruiru 11) were evaluated. The Uganda tetraploids used in generating the interspecific hybrids were sourced from Uganda while the Robusta and Arabica genotypes are all from Coffee Research Institute-Ruiru, Kenya.

The trials were established at Siaya ATC (Siaya County) and KALRO Alupe (Busia County) both of which sites are located near the Lake Victoria basin in the low altitude zones suitable for planting Robusta coffee. Siaya lies between 0° 30 N′ and 0° 45′ E with an altitude that varies from 1,135 m to 1,500 m above sea level receiving a mean annual rainfall of 1,500 mm whereas Busia county lies between 0° 30 N′ and 34° 30' SE with an altitude that varies from 1241 m to 1343 m above sea level with mean annual rainfall of 1400 mm.

3.2 Sensory evaluation of coffee

The evaluation of the sensory attributes was conducted by five trained judging panel using the procedures described by [64, 65]. A probate laboratory roaster was used in the roasting process and the roasted beans were left to rest for at least 8 hours before cupping. Green coffee beans were weighed before and after roasting

to be able to determine the roasting degree. After the 8 hours', the roasted beans were ground into individual cups ensuring that the whole sample was deposited into each cup. Each sample representing a specific genotype was placed into five cups. Samples were weighed to get 8.25 g and 150 ml of hot water was added per cup. The evaluation of the sensory attributes was conducted by five trained judges forming a panel using the procedures described by [65]. The descriptors measured included acidity, body, balance, fragrance/aroma, flavour, aftertaste, and preference as described by SCA.

The attribute scores of clean cup, sweetness, and uniformity were each scored and a maximum of two points per cup was awarded getting a maximum score 10. These scores were added to the scores obtained from the other seven sensory attributes to constitute the total score. This would then reflect the total performance of genotypes regarding cup quality. The average score of a cupper was considered as a replication.

3.3 Biochemical compounds analyses

3.3.1 Extraction and quantification of crude oil

Two (2) grams of the dried green coffee powder from the green coffee bean was weighed and dried for 1 h at 105°C ± 2°C. Extraction was carried out after adding 100 mm of hexane to the coffee powder which was then in the soxhlet extraction apparatus [66]. Rota vapor was used to dry the extract and placing it an oven at105 ± 2°C to complete drying process. The extract was cooled and then weighed to get the final weight after evaporation. The drying process continued for another two hours weighing being undertaken at a 30-minute interval until there was no more than one milligram loss between successive weighing. Crude oil content was then calculated by as the increase in weight of the extraction flasks [67].

3.3.2 Extraction of caffeine, trigonelline and total chlorogenic acids (CGA)

Caffeine, trigonelline and chlorogenic acids levels were determined using the protocols as provided by [68, 69] with slight modifications as described below.

3.3.3 Analysis of caffeine, trigonelline and total chlorogenic acids

HPLC system (Knaeur) equipped with a Super Co Discovery C-18 column was used to analyse caffeine and trigonelline and BDS HYPERSIL C-18 column used to analyse chlorogenic acids. Diode Array Detector was used to detect the three wavelengths, at 278 nm for caffeine, 266 nm for trigonelline and 324 nm for CGA. HPLC grade methanol (PANREAC) 35% was used as the mobile phase, distilled water 65%, acetic acid (PROLABO) 0.1%, at a flow rate of 1 ml/min under ambient temperature. The retention times of the trigonelline standard (Sigma Aldrich), CGA standard (Acros organics) and caffeine standard (99%) (Fischer Scientific) were used to calculate trigonelline, CGA and Caffeine quantities respectively. Calibration equations were used to calculate using the peak area of the slope [67].

3.3.4 Extraction and analysis of sucrose

The extraction and analysis of sucrose was done according to the method of [70] used by [67]. 0.2 g of the green coffee powder was added to 100mls of 96% ethanol under reflux. The extract was evaporated to dryness after filtering it using the Whatman filter paper number 42. Recovery of sucrose was done using 10mls

deionized water and 2mls of the extract mixed with 2mls Diethyl ether (AR) and the top layer was discarded after settling. The process was repeated three times and 1 ml of acetonitrile was added to 1 ml of the extract. Filtering was conducted using the 0.45 µm micro filter. HPLC system (Knaeur) equipped with a Eurospher 100–5 NH2 column and a refractive index detector was used to analyse sucrose. Acetonitrile HPLC grade (SCHARLAU) 75%, and distilled water 25% was used as the mobile phase at a flow rate 1 ml/min. The sucrose standard (Fischer Scientific) was used in quantifying the sucrose level through comparison of the retention peak of standards and sample peak the sucrose level calculated using the calibration equation.

3.4 Data analysis

The bean grades, sensory data and biochemical data were subjected to Analysis of Variance (ANOVA) using GENSTAT statistical software version 18 and effects declared significant at 5%. The General Linear Model (GLM) was used.

$$Y\hat{} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + E_i. \quad (1)$$

Where,

For each observation $i = 1, \ldots, n.$ where n is the observations of one dependent variable.

$Y\hat{} = j^{th}$ observation of the dependent variable.

$j = 1,2, \ldots, k.$

X = is the observation of the j^{th} independent variable.

β = parameters to be estimated.

Ei = Distributed normal error.

Least Significance Difference was used to separate means [71]. Separate as well as combined analysis of variance was performed on data from the two sites. GENSTAT statistical software was used to compute correlation and to show relationship between sensory traits using the Pearson Correlation Coefficient.

3.5 Sensory performance

Sensory traits significantly varied among the coffee genotypes tested across the two locations with Arabica genotype SL28 recording the highest Fragrance value and Robusta genotypes the lowest. Again as for Flavour, Arabica genotype, SL28 recorded the highest value whereas CV1 recorded the lowest (**Table 1**). Again, genotype SL28 recorded significantl higher values for Aftertaste in both sites. As for Acidity, Robusta genotypes had the lowest values but Arabica genotype SL 28 recorded the highest. Body value was high in both Arabusta hybrids and Arabica genotypes. For all the traits scored, Arabica genotype, SL28 recorded significantly higher values than all the rest, across the two locations (**Table 2**).

The genotypic effect varied significant for all the sensory traits with the exception of the environmental variations were significant for all the sensory trait whereas the G x E interaction was not significant for all the sensory traits measured (**Table 2**). Preference scored the highest maximum score, whereas acidity scored the lowest. (**Table 3**). The highest rated sensory attribute was Body, followed closely by Aroma whereas Flavour and Aftertaste had the lowest mean. Acidity and preference indicated that they had wider phenotypic variance than all the other sensory traits (**Table 3**).

Genotypes	Fragrance		Flavor		Aftertaste		Acidity		Body		Balance		Preference		Total score	
	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si	Bu	Si
ARH1	7.5	7.0	7.4	7.0	7.6	7.1	7.4	7.2	7.7	7.5	7.4	7.2	7.4	7.1	82.4	80.1
ARH4	7.4	7.5	7.2	7.0	7.4	6.9	7.2	7.0	7.6	7.4	7.3	7.0	7.4	7.0	81.5	79.8
ARH5	7.8	7.3	7.5	7.2	7.7	7.1	7.5	7.1	7.8	7.5	7.6	7.1	7.6	7.2	83.5	80.5
ARH6	7.5	7.4	7.3	7.3	7.4	7.3	7.4	7.4	7.5	7.6	7.6	7.4	7.5	7.4	82.2	81.8
ARH7	7.6	7.6	7.5	7.4	7.7	7.2	7.6	7.4	7.7	7.7	7.7	7.3	7.7	7.4	83.5	82.0
BC01	7.4	7.4	7.2	6.9	7.3	6.8	7.3	6.9	7.6	7.4	7.4	7.0	7.4	6.9	81.6	79.3
BC02	7.5	7.5	7.3	7.2	7.3	7.1	7.3	7.0	7.5	7.5	7.2	7.2	7.3	7.1	81.4	80.6
BC03	7.8	7.7	7.1	7.3	7.3	7.3	7.3	7.5	7.5	7.5	7.2	7.4	7.4	7.4	81.6	82.3
BC04	7.6	7.5	7.4	7.2	7.5	7.2	7.5	7.4	7.5	7.5	7.4	7.1	7.4	7.2	82.3	81.2
BC05	7.7	7.4	7.6	7.3	7.6	7.1	7.6	7.4	7.7	7.5	7.8	7.4	7.7	7.4	83.7	81.6
BC06	7.6	7.4	7.4	7.0	7.4	6.8	7.5	6.8	7.6	7.5	7.4	7.0	7.4	6.9	82.3	79.0
CV1	7.6	7.1	7.2	6.7	7.4	6.6	7.2	6.7	7.6	7.5	7.4	7.3	7.3	6.6	81.7	78.2
CV2	7.4	7.2	7.2	6.8	7.4	6.9	7.2	6.9	7.5	7.5	7.4	7.0	7.3	6.7	81.4	78.7
ARV	7.4	7.6	7.4	7.3	7.5	7.3	7.4	7.4	7.7	7.5	7.4	7.4	7.4	7.4	82.2	82.0
Robusta	6.8	6.9	7.2	7.0	7.1	7.1	7.0	6.9	7.2	7.5	7.0	7.0	7.1	7.0	79.5	79.1
Ruiru 11	7.7	7.2	7.3	7.0	7.3	7.0	7.5	7.2	7.5	7.5	7.3	7.4	7.4	7.3	82.0	80.6
Batian	7.6	7.9	7.5	7.9	7.3	7.9	7.4	8.1	7.6	7.5	7.2	7.2	7.2	7.2	81.8	83.8
SL28	8.1	8.2	7.9	8.2	8.0	8.1	7.8	8.2	7.9	7.5	7.9	7.9	8.3	7.9	85.9	86.2
LSD	0.3	0.4	0.3	0.5	0.3	0.4	0.3	0.4	0.4	7.5	0.3	0.6	0.3	0.4	1.5	2.4
%CV	0.7	3.6	1.8	2.3	2.5	2.4	2.0	2.7	1.6	7.5	1.1	3.1	1.5	1.9	0.7	0.9
Ftest	S	S	S	S	S	S	S	S	NS	7.5	S	S	S	S	S	S

Key: Bu- Busia Si- Siaya; Reproduced from PhD thesis, University of Nairobi.

Table 1.
Sensory traits for coffee genotypes at KALRO-Alupe and Siaya ATC.

Source	Rep	Gen (G)	Envt (E)	G x E	Error
Df	4	17	1	17	140
Fragrance	0.598	0.3514***	0.73472**	0.12296NS	0.096
Flavour	0.152	0.6629***	2.6889***	0.0793NS	0.102
Aftertaste	0.151	0.4416***	7.4014***	0.0911NS	0.106
Acidity	0.213	0.7609***	2.6281***	0.1524NS	0.113
Body	0.536	0.1769***	0.6183***	0.0926NS	0.102
Balance	0.202	0.3225NS	2.4019***	0.1402NS	0.159
Preference	1.218	21.18***	134.421***	4.525NS	2.882

*Key: *, **, *** and NS represent significant at (P < 0.005), (P < 0.001), (P < 0.0001) and non-significant respectively. Reproduced from PhD thesis, University of Nairobi.*

Table 2.
Mean squares for sensory traits of 17 coffee genotypes evaluated at Siaya ATC and KALRO-Alupe (Busia).

Attributes	Minimum	Maximum	Mean	Variance range	Standard Error
Aroma	7.23	8.00	7.48	0.78	0.09
Flavor	6.93	8.00	7.28	1.08	0.10
Aftertaste	6.98	7.88	7.28	0.90	0.10
Acidity	6.90	8.08	7.31	1.18	0.10
Body	7.30	7.83	7.53	0.53	0.10
Balance	7.15	7.85	7.34	0.70	0.13
Preference	6.93	8.10	7.32	1.18	0.09

Reproduced from PhD thesis, University of Nairobi.

Table 3.
Variability of the sensory attributes for the 20 coffee genotypes.

The biochemical attributes scored here, varied significantly among the genotypes with genotypes ARH2 and ARH3 scoring the highest levels of chlorogenic acids, caffeine, sucrose and Trigonelline contents (**Figure 1**). Arabica genotypes, Ruiru 11, Batian and SL28 gave the highest oil content values, whereas Robusta recorded the highest caffeine contents (**Figure 1**). In the two locations over the two seasons, there was variation in the biochemical composition of the Arabusta hybrids, backcrosses, Robusta and Arabica coffee genotypes evaluated here. Arabica coffee genotypes had the highest composition of sucrose, trigonelline and oils, whereas the Arabusta hybrids scored intermediate values between Arabica and Robusta. Robusta genotypes scored the highest caffeine and cholorogenic acid contents whereas Arabica scored the lowest (**Figure 2**).

The Arabusta hybrids had higher values of oil, sucrose and trigonelline contents than Robusta genotypes which contributed to a better cup quality. As noted elsewhere in this chapter, chlorogenic acids are involved in aroma formation and pigmentation of coffee whereas caffeine influences the mildness in the cup [72]. But higher levels of caffeine and chlorogenic acids lower the quality by infusing bitterness and the astringency taste in the coffee brew [64, 73]. The results reported here showed that, Arabica and Arabusta genotypes had higher levels of sucrose, oil and trigonelline contents than Robusta genotypes, that contributed to a better cup quality due to the aroma and flavor that these biochemical compounds produce. All the interspecific

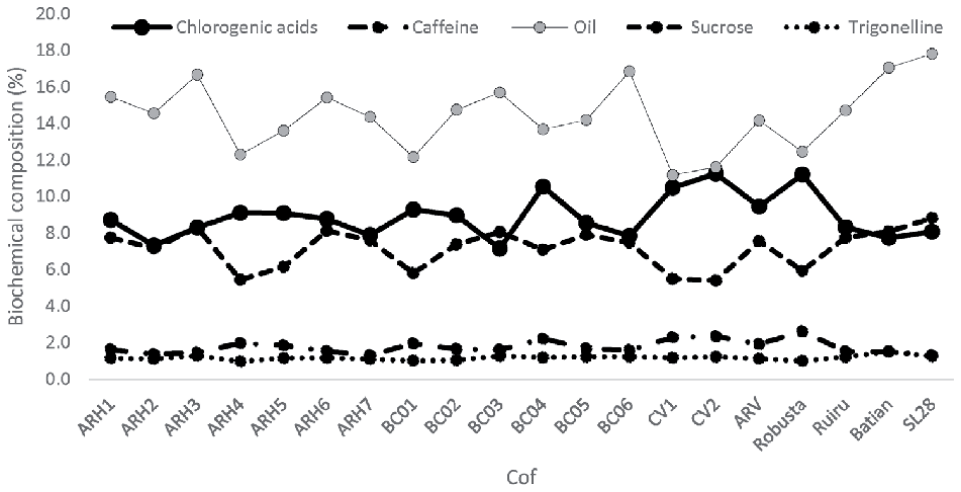

Figure 1.
Biochemical contents of twenty coffee genotypes in Busia and Siaya. Reproduced from PhD thesis, University of Nairobi.

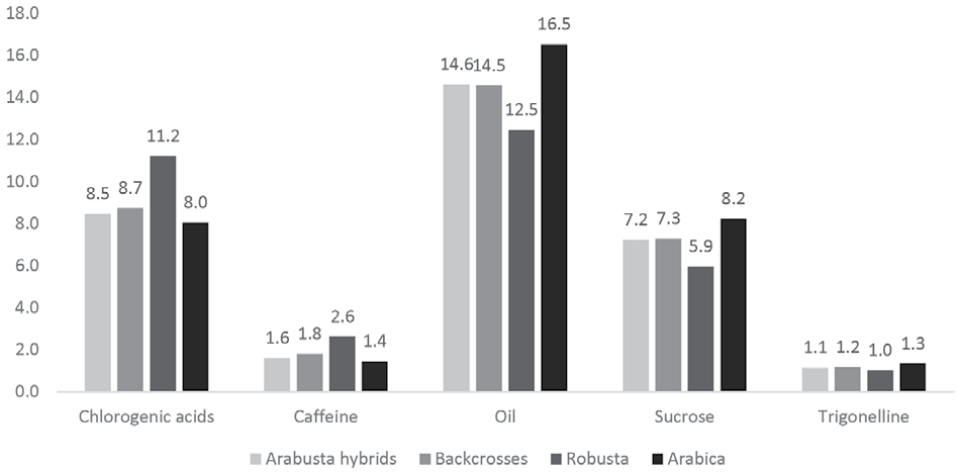

Figure 2.
Biochemical composition for the Arabusta coffee hybrids, Backcrosses, Arabica and Robusta coffee. Reproduced from PhD thesis, University of Nairobi.

hybrids with the exception of ARH4 genotype recorded a 80% quality performance compared to Robusta genotypes.

4. Conclusions

Arabica and Arabusta genotypes evaluated in these experiments, confirmed that there is genetic variation for organoleptic, sensory and biochemical traits in coffee.

Interspecific hybridization between *C. Arabica* and *C. canephora*, produced hybrids with improved sensory and organoleptic traits that were intermediate between the two species. Cup quality in coffee can be improved through selection and hybridization in coffee improvement programs.

Author details

Kahiu Ngugi[1*], Jane Jerono Cheserek[2] and Chrispine Ogutu Omondi[3]

1 Department of Plant Science and Crop Protection, Faculty of Agriculture, College of Agriculture and Veterinary Sciences, University of Nairobi, Nairobi, Kenya

2 Coffee Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO), Ruiru, Kenya

3 Sugar Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO), Kisumu, Kenya

*Address all correspondence to: kahiukahiu@gmail.com

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Section 3

Calcium and Bone Metabolism

Chapter 5

Parathyroid Glands and Hyperparathyroidism: A General Overview

Andre Luis Maion Casarim

Abstract

Hyperparathyroidism (HPT) is a clinical condition caused by the increase of parathyroid hormone (PTH) synthesis by the parathyroid glands. PTH has a central and fundamental role in the control of calcium and phosphorus homeostasis. Its action on the kidney, bone, and, indirectly, intestinal cells implies a rapid increase in extracellular calcium flow. This clinical condition may be due to an intrinsic parathyroid disorder or secondary to an imbalance of calcium metabolism in patients with systemic diseases, such as chronic renal failure. The treatment of hyperparathyroidism may be clinical, with the control of calcium, phosphorus, and PTH levels, or surgical, depending on the various forms presented. The purpose of the chapter is to discuss the types of hyperparathyroidism, their relationship with phosphorus and mainly calcium metabolism, as well as the main forms of diagnosis and treatment.

Keywords: hyperparathyroidism, calcium, bone diseases, kidney diseases, parathyroid hormone

1. Introduction

Hyperparathyroidism (HPT) is a pathology caused by the increased synthesis of parathyroid hormone (PTH) by the parathyroid glands. This process can be a consequence of an intrinsic parathyroid disorder or secondary to an imbalance of calcium metabolism in patients with systemic diseases, such as chronic kidney disease (CKD) [1]. PTH plays a central and fundamental role in homeostasis of the control of calcium and phosphorus in the body. Its action on the renal, bone, and, indirectly, intestinal cells implies a rapid increase in the extracellular flow of calcium. This hormone has a short half-life (2–3 min) that quickly mobilizes calcium to the intravascular. It binds to specific membrane receptors on the kidney and bone cells, fibroblasts, chondrocytes, vascular smooth muscle, adipocytes, and placental trophoblasts [2].

2. History

The parathyroid glands were initially discovered in the eighteenth century by Richard Owen, who dissected the parathyroid glands of an approximately 2260 kg

rhino, being reported as "a small, compact yellow glandular body attached to the thyroid at the point where the veins emerge" [3, 4]. However, the definitive discovery of the parathyroid glands in humans was made in 1877 by Swede Ivar Sandström, a medical student at Uppsala University, Sweden, publishing his work in which he wrote: "Almost three years ago I found on the thyroid gland of a dog a small organ, hardly as big as a hemp seed, which was enclosed in the same connective tissue as the thyroid, but could be distinguished therefrom by the light color. Microscopically the examination revealed glandular tissue completely different from that of the thyroid" [4]. After identification in dogs, he finally dissected such structures in human cadavers, calling them *glandulae parathyroidae* (**Figure 1**).

Later, in 1891, Eugene Gley reported symptoms of tetany when these glands were removed during thyroidectomies [5]. At the beginning of the twentieth century, the theory described by Jacob Erdheim emerged, which related hypertrophy of parathyroid tissue as a response to bone disease (osteomalacia and/or cystic fibrous osteitis). This theory was rejected by Felix Mandl in 1925, who performed the first parathyroid surgery in Vienna. Initially, it was believed that bone lesions were a consequence of a parathyroid deficiency, and to prove it, transplantation of cadaveric parathyroid tissues was performed in patients with cystic bone lesions and hypercalciuria. With the procedure unsuccessful, Mandl proceeded with cervical exploration and identified a 21 × 12 × 12 mm parathyroid "tumor," which was resected, observing the patient's improvement [6]. Still in 1925, Collip brought a breakthrough in studying the function of PTH, by treating patients with tetany due to parathyroidectomy with relative PTH extract with relative success [4].

The term tertiary HPT came up with Dr. Walter St. Goar, when describing a case report in the New England Journal of Medicine, number 268, in 1963, of a patient with CKD and functional parathyroid autonomy [7]. McPhaul, in 1964, published

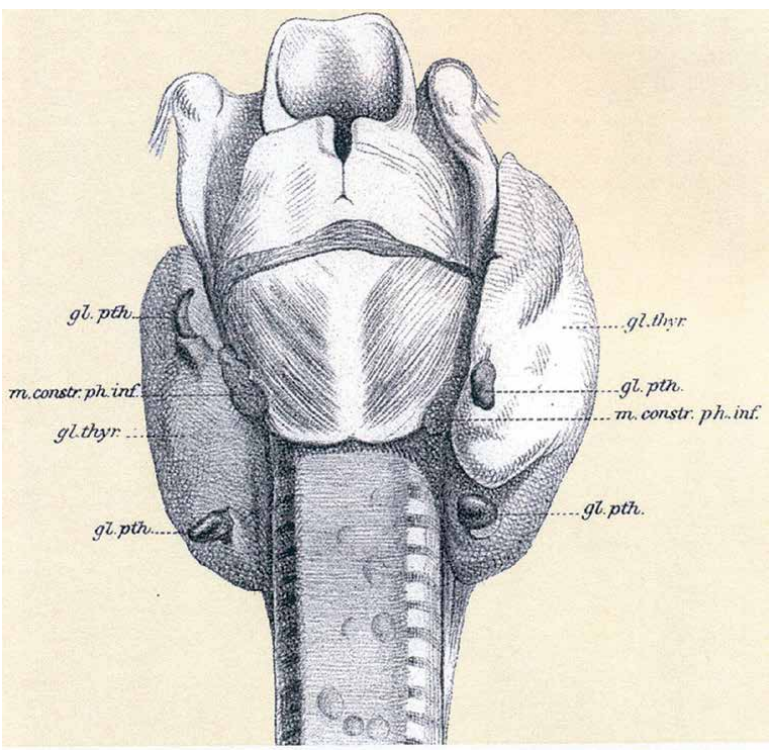

Figure 1.
Original drawing by Ivar Sandström of human parathyroid glandular anatomy [4].

the first surgical success in patients with this clinical condition [8]. Later, in 1968, Davies and colleagues also studied 200 cases of HPT and, of these, obtained 14 cases of tertiary HPT [7].

3. Physiology

PTH plays a central and fundamental role in homeostasis of the control of calcium and phosphorus in the body. It is produced by the parathyroid glands, composed mainly of main cells, and, to a lesser extent, by oxyphil cells [1, 4]. Studies show that, over time, the main cells are replaced by oxyphil cells, which are more sensitive to PTH production, especially in cases of CKD [9, 10]. Its action on renal, bone, and, indirectly, intestinal cells implies a rapid increase in the extracellular flow of calcium (**Figure 2**). This hormone is a peptide with 84 amino acids and a molecular weight of 9500 Da. It has a short half-life (2–3 min) and is broken down into the amino-terminal and carboxy-terminal portions. Only the amino-terminal portion has biological effects on the body, while the carboxy-terminal portion remains inactive. PTH binds to specific membrane receptors in renal and bone cells, fibroblasts, chondrocytes, vascular smooth muscle, adipocytes, and placental trophoblasts [11].

3.1 Effect of PTH on the kidneys

PTH causes an increase in the calcium reabsorption of the glomerular filtrate. This is mainly due to some factors. First, it acts in the loop of Henle, increasing the voltage gradient to allow the passive transport of ionized calcium. Then, it acts on the granular portion of the distal contorted tubules, inducing translocation of preformed calcium channels on the cell surface, increasing the entry of calcium into the vascular lumen. Finally, it also acts on the collecting tubules by changing the activation of the Na^{+}/Ca^{2+} pump [11–13]. In addition, PTH has other effects on kidney cells, such as an increase in phosphate excretion, with increased phosphaturia and decreased serum phosphate; increased bicarbonate clearance with urine

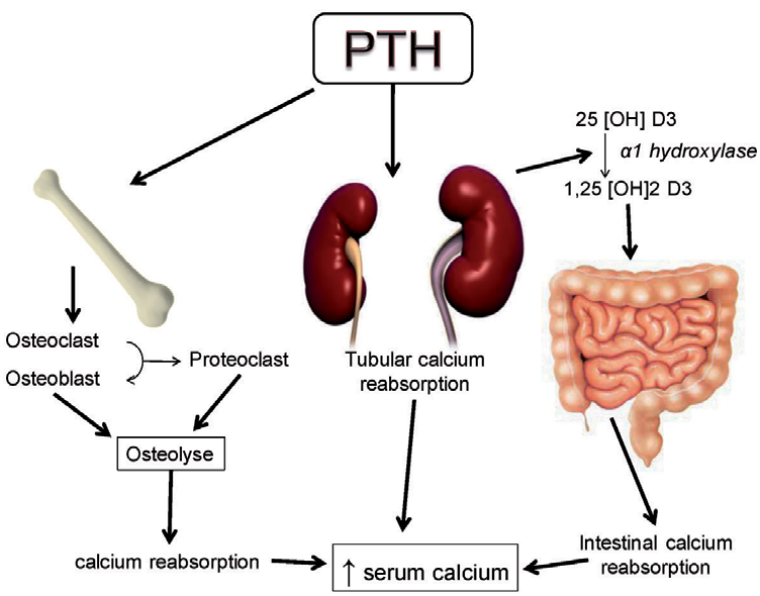

Figure 2.
Mechanisms of action of parathyroid hormone.

alkalinization, resulting from decreased bicarbonate reabsorption in the proximal renal tubules; inhibition of sodium reabsorption in the proximal tubules, with increased clearance of free water and greater urinary flow; and increased activity of vitamin D1 alpha-hydroxylase, with greater production of the active form of vitamin D (calcitriol) [12, 14, 15].

3.2 Effect of PTH on bones

PTH produces both anabolic and catabolic effects, depending on the phases of action. In the early phase, there is a mobilization of calcium from the bones, entering equilibrium with the extracellular fluids. In the late phase, there is an increase in the synthesis of bone enzymes, such as lysosomal enzymes, which promotes bone resorption and remodeling. PTH also inhibits osteoclasts and stimulates bone resorption, leading to an increase in serum alkaline phosphatase and urinary hydroxyproline (markers of osteolysis) [11–13].

3.3 Effect of PTH on the intestine

PTH has no direct action on the intestine. Its effect is due to an increase in the synthesis of calcitriol (activate form of vitamin D) in the kidneys, through the stimulation of the enzyme α1-hydroxylase. Calcitriol has an effect on increasing serum calcium from the resorption of bowel lumen [11].

4. Embryology and anatomy of the parathyroid glands

Embryologically, the parathyroid glands have an endodermal origin, usually four glands, and rarely may be in number of three, five, or more glands. They develop through the proliferation of the dorsal part of the branchial pharyngeal pouches. Both parathyroid glands migrate to the posterior portion of the thyroid gland, since the lower ones are able to migrate to the thymus or upper mediastinum [4, 12, 16].

4.1 Inferior parathyroid glands

The inferior parathyroid glands originate from the dorsal portion of the third branchial pouch. This branchial pouch differs around the 5th to the 6th week of gestation, when the ventral portion gives rise to the thymus. Around the 7th week of pregnancy, both the thymus and the lower parathyroid loosen the connection with the pharynx, occurring medial and caudal migration of the thymus, bringing together the inferior parathyroid, when, finally, it separates from the thymus. Usually the inferior parathyroid glands are found outside the capsule of the thyroid gland and have a more variable location [17]. Caudal migration close to the thymus explains the lower localization of the inferior parathyroid glands (derived from the third branchial pouch) than the superior parathyroid glands (derived from the fourth branchial pouch). During this process, debris from the parathyroid tissue that can form supernumerary glands may detach. The arterial supply of the inferior parathyroid glands is from the branches of the inferior thyroid artery [17–19].

4.2 Superior parathyroid glands

The superior parathyroid glands originate from the dorsal portion of the fourth branchial pouch. In the 7th week of pregnancy, they lose their relationship with the

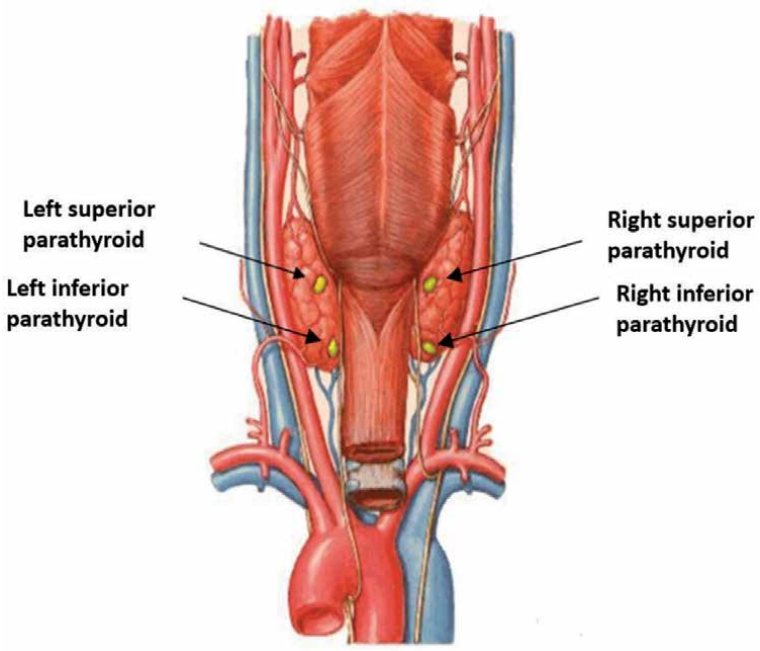

Figure 3.
Anatomical location of the parathyroid glands, posterior view (modified by F. netter, atlas of human anatomy).

pharynx and adhere to the thyroid gland, which is migrating caudally and medially. This migration occurs to a lesser extent than the migration of the thymus and inferior parathyroid glands, and therefore their locations are more predictable. Superior parathyroid glands are usually located more posteriorly and medially than inferior parathyroid glands, often located on the dorsal surface and outside the thyroid capsule [4, 17–19].

These glands are closely related to the inferior thyroid artery and its intersection with the recurrent laryngeal nerve, although many anatomical variations may occur. The superior parathyroid glands have arterial supply from the branches of the inferior thyroid artery and posterior branches of the superior thyroid artery. The inferior thyroid artery generates branches to supply the parathyroid glands before irrigating the thyroid lobes.

Parathyroid appears in the body as two superior and two inferior glands in approximately 85–90% of the population. In addition, in 10% of the population, they appear as supernumerary, with 5 or more glands, with reports of up to 10 parathyroid glands in a single patient [19].

Figure 3 shows the locations of the parathyroid glands.

5. Clinical presentations

HPT can be divided into primary, secondary, or tertiary.

5.1 Primary HPT

PTH, in these cases, is produced through a stimulus of the parathyroid due to an intrinsic pathology of this gland. In 85% of the cases, a parathyroid adenoma forms, generating an increase in PTH production. However, hyperplasia of the

parathyroid gland (13%) or carcinoma (1%) can occur. Parathyroid adenoma is the most common clinical presentation in primary HPT. It usually presents with only one adenoma, but it can manifest in more than one parathyroid, known as multiple adenomas [20–22]. Structural genetic mutations are associated with the etiology of the adenoma, although it can also occur after exposure to neck irradiation, treatment with lithium, or thiazide diuretics [20, 23]. The consequence is the disorderly overproduction of PTH, increasing osteoclastic activity and therefore raising calcium levels in the body. PTH also stimulates renal calcium reabsorption and acts on the activation of vitamin D, increasing intestinal calcium absorption. Most are asymptomatic; however the patient may develop clinical manifestations. The most frequent consequence of primary HPT is nephrolithiasis that develops in almost 15–20% of cases. Nevertheless, HPT is found in just 5% of patients with nephrolithiasis. Osteoporosis, fibrous osteitis, and peptic disease may be also found in these patients. Neuropsychiatric and neuromuscular disorders such as asthenia, weakness, and mild depression can be found less frequently [24]. In rare presentations, brown tumors may be formed, the etiology of which is associated with the disruption of bone turnover between osteoblastic and osteoclastic activity, resulting in benign bone tumors. Sporadic presentation is more frequent; however, family forms may be associated. The main familial manifestations are multiple endocrine neoplasia (MEN) type I, or Werner's syndrome (HPT, pancreatic tumor and pituitary tumor), and type IIA, or Sipple's syndrome (HPT, medullary thyroid carcinoma and pheochromocytoma), and are associated with the expression of the RET proto-oncogene [24, 25].

Other existing familial forms described are primary neonatal HPT, familial hypocalciuric hypercalcemia, jaw tumor HPT syndrome, and isolated familial HPT [26]. Associated with primary HPT is the parathyroid carcinoma, a rare presentation, less than 1% of cases. It is suspected when there are high levels of serum calcium and PTH and may also have a palpable cervical mass on the thyroid gland topography. HPT in patients, if not treated quickly and efficiently, can progress rapidly to complications secondary to hypercalcemia and may progress to death [20, 26, 27].

5.2 Secondary HPT

Secondary HPT, unlike primary HPT, is a systemic and non-intrinsic pathology of the parathyroid gland, the consequence of which affects the functioning and metabolism of the parathyroid glands. It is the result of a parathyroid response to a tendency of hypocalcemia in order to maintain calcium homeostasis. It occurs due to low calcium absorption and vitamin D deficiency. Hypovitaminosis D is the main cause of secondary HPT in developed countries, in which confinement and low sun exposure occur frequently. As a result, there is a decrease in intestinal calcium absorption and a tendency towards serum hypocalcemia. The feedback mechanism stimulates the parathyroid glands and increases PTH synthesis, mobilizing calcium from the bones to maintain intravascular calcium homeostasis. Another important cause of secondary HPT is CKD, in which the kidney injury generates low calcium reabsorption in the distal renal tubules with consequent hypocalcemia. It is also in the kidney that the conversion of vitamin D (cholecalciferol) to its active form (calcitriol) occurs through the enzyme alpha1-hydroxylase. In CKD, this conversion is impaired and shows a consequent decrease in serum calcitriol rates and intestinal calcium absorption. As a result, there is an increase in PTH production and osteoclastic activity to try to normalize serum calcium levels. In advanced cases, there is intense damage to bone health, with osteoporosis, severe bone pain, fibrous osteitis, and even pathological fractures [16, 28, 29]. At the same time, renal injury causes phosphate retention, with an increase in serum inorganic phosphate.

The chronic renal patient with secondary HPT frequently presents with major bone disease, bone pain, itching, cardiovascular disorders, and, in advanced cases, pathological bone fractures [28, 29].

5.3 Tertiary HPT

Tertiary HPT manifests itself through an evolution of secondary HPT. In these cases, the continuous stimulus to the overproduction of PTH leads the autonomy of the parathyroid glands to produce PTH in high amounts. In the case of CKD, many patients undergo kidney transplants, expecting normalization of calcium reabsorption, conversion of vitamin D to its active form (calcitriol), and a consequent drop in PTH levels and normalization of the calcium rates. However, autonomous parathyroid glands maintain PTH overproduction even with renal calcium reabsorption normalized. The main consequence is the increase of the calcium serum levels. Most patients are asymptomatic, although some maintained bone pain and risk of fractures. In these cases, persistent hypercalcemia, chronic renal graft dysfunction, bone disease progression, cardiovascular events, and the risk of developing nephrolithiasis in the transplanted renal graft justify the early treatment of the disease [7, 30].

6. Diagnosis

In HPT, anamnesis and physical examination are of fundamental importance for accurate diagnosis.

Hypercalcemia is the main consequence of the primary HPT. In most cases, the disease is asymptomatic, occupying more than 80% of cases [21, 26, 31]. This condition was introduced after the 1970s, when routine laboratory tests began to be performed in asymptomatic patients [32]. However, they can develop symptoms that usually begin when calcium levels exceed 12 mg/dL and include manifestations in several systems. Neurological disorders manifest with changes in the level of consciousness, confusion, or lack of concentration. Gastrointestinal manifestations, such as nausea, epigastric pain due to peptic disease, or even pancreatitis, can be found. Nephrolithiasis can occur, mainly of repetition, polydipsia or polyuria. Bone pain and pathological fractures are also reported, in addition to brown tumors in the bone skeleton. Cardiovascular manifestations and heart rhythm disorders are also associated with hypercalcemia [33]. We can measure ionized serum calcium or total calcium. The total calcium measurement needs to be adjusted, because much of it is bonded with serum albumin. The formula can be shown in the following: corrected calcium = measured total serum calcium in mg/dL + 0.8 × (4.0 − patient's serum albumin concentration in g/dL) [21]. Vitamin D participates actively in the diagnosis of primary HPT. The Institute of Medicine (IOM) affirms an evidence that the disease is more active when the vitamin D levels are below normal [21, 34]. A variant of primary HPT is the normocalcemic HPT, when presents with levels of PTH above normal and normal levels of serum calcium. The evolution of these cases can be stable, without clinical complications or laboratory alterations, increase the serum calcium concentration, or cause bone, kidney, or cardiovascular impairments [35].

Secondary HPT caused by CKD normally courses with normal or low levels of serum calcium. Thus, the history of CKD, especially dialysis, is fundamental for the diagnostic interpretation. The symptoms are due to bone disease with bone pain that can be of different intensities and pathological fractures with difficulty in movement. The pruritus also is most frequent, especially when the phosphate levels are above the normal.

Laboratory assessment of HPT is essential for diagnosis. Serum measurements of PTH, total serum calcium, ionized fraction of calcium, phosphorus, vitamin D, and alkaline phosphatase should be ordered. PTH will always be increased but to varying degrees. In primary HPT, PTH normally increases from two to four times normal, but it can reach much higher values depending on the severity of the disease. Consequently, serum calcium also rises. Phosphorus levels are normal or low and alkaline phosphatase rises [33]. Very high levels of PTH and calcium raise the hypothesis of parathyroid carcinoma and require a different approach. In secondary HPT there is a high elevation of PTH, commonly reaching the values of 10–20 times higher than normal or above in several cases. Calcium is usually in the normal range or at reduced levels, with hyperphosphatemia occurring in some cases. Due to decreased vitamin D activation in the kidney, there is a decrease in serum calcium due to low intestinal absorption.

In tertiary HPT, the parathyroid glands become autonomous in the production of PTH, despite the improvement in renal function, observed mainly in post-kidney transplant patients [36]. There is not a sufficient decrease in PTH, which remains in the range of 5–10 times greater than normal. Vitamin D levels also tend to normalize, since the absence of kidney damage in the post-transplant patient normalizes the conversion of vitamin D into its active form (calcitriol) and also normalizes the intestinal absorption of calcium. Thus, the mechanisms lead to increased serum calcium concentration [36, 37].

Complementary imaging exams are of great value in therapeutic planning, especially when the surgical approach is programmed [38].

Cervical ultrasonography has an important value, i.e., in trying to locate the enlarged parathyroid glands, although there is difficulty due to the overlying position of the thyroid gland and the fact that it is operator dependent. Also, we need to study the thyroid anatomy and the possibility of thyroid nodules that can be approached at the same time, if surgery is performed [39, 40].

Computed tomography, as well as magnetic resonance imaging of the neck, can also help in locating the altered glands, especially if they are much larger than normal. One of the main exams is parathyroid scintigraphy, as it can functionally reveal parathyroid glands with increased activity through the uptake of 99Tc-sestamibi (MIBI) [38, 39]. Even more sensitive and specific than scintigraphy is SPECT/CT with MIBI. This examination has the same physiological foundations as flat scintigraphy; however its fusion with the computed tomography image allows for a much more precise location of the affected parathyroid glands.

Another resource is 4D computed tomography. The mechanism of realization of this exam is based on the arterial supply and venous drainage of the analyzed structure. It helps to differentiate a parathyroid gland from a lymph node or any other structure present in the neck or mediastinal region [38, 40, 41].

7. Treatment

The treatment for HPT may be with clinical management or surgery. However, there are other previous forms of disease control, mainly in primary asymptomatic HPT and secondary HPT in early stages. In asymptomatic cases of primary HPT, clinical follow-up without surgical treatment can be performed in most cases. However, in symptomatic cases with evidence of bone and kidney disease, surgical treatment is preferred [27, 31, 32, 42]. Treatment with bisphosphonates and calcimimetics, such as cinacalcete, is applied in cases of severe hypercalcemia and difficulty in performing surgical treatment in a timely manner [43, 44]. Therapeutic doubt arises in asymptomatic cases, in which initial surgical treatment is often not

indicated. According to the Fourth International Workshop, held in Italy in 2013 [21], surgical indications in asymptomatic patients are reserved for the situations illustrated in **Table 1**.

The surgery performed on primary HPT is, in most cases, resection of the parathyroid affected by the adenoma (85% of cases). The use of intraoperative rapid PTH allows less chance of persistent disease (as in multiple adenomas) or future recurrences [26, 45–47]. In cases of parathyroid hyperplasia in primary HPT, as in multiple endocrine neoplasia, the surgical approach is the subject of much discussion. The possibility of subtotal/partial parathyroidectomy or total parathyroidectomy with autograft of fragments of parathyroid tissue in muscle tissue (forearm, presternal, or deltoid musculature) is described [25, 48]. Although rare, being responsible for less than 1% of cases of primary HPT, parathyroid carcinoma, when clinically suspected, should be treated with special care. Cases of severe hypercalcemia (greater than 14 mg/dL) and high levels of PTH (15–20 times higher than normal) should have parathyroid carcinoma as a diagnostic hypothesis. The recommended treatment is resection of the tumor en bloc with wide safety margins. The complete surgery includes partial thyroidectomy ipsilateral to the affected parathyroid, lymphadenectomy of the central compartment, and thymectomy [49].

In secondary HPT, initial clinical treatment is focused on the control of calcemia and phosphatemia and reduction of factors that stimulate PTH overproduction, as well as avoiding exposure to aluminum. Administration of vitamin D and calcium carbonate is used to try to correct serum calcium levels. Surgical treatment is usually indicated when intact PTH levels exceed 800 pg./mL, or when persistent symptoms such as bone fractures, pain refractory bone, intractable pruritus, brown tumor, and ectopic calcification. The pathology is systemic, and hyperplasia of all parathyroid glands occurs through feedback mechanisms. Therefore, the surgery to be performed can be the total parathyroidectomy with autograft of fragments of parathyroid tissue in the muscle tissue (forearm, presternal, or deltoid musculature) in order to avoid persistent hypoparathyroidism, or subtotal parathyroidectomy, with maintenance of parathyroid tissues to avoid persistent hypoparathyroidism [50–52]. The clinical management of secondary HPT is increasingly available, with acceptable responses, especially in patients who are not candidates for surgical treatment. Calcimimetics, such as cinacalcet, have acceptable results in reducing serum PTH and, mainly, improving the quality of life of chronic renal patients. Tolerability is reasonable, with some side effects, such as nausea, vomiting, and hypocalcemia, which may suspend treatment in some cases [53].

Indication of parathyroidectomy in asymptomatic primary HPT	
1. Serum calcium values	Serum calcium 1.0 mg/dL (0.25 mmol/L) greater than the upper limit of normal
2. Imaging findings	A. Bone mineral density: T-score < −2.5 in the lumbar spine, hip, femur, or distal third of the radius
	B. Vertebral fracture on X-ray, CT, MRI, or bone densitometry
3. Renal factors	A. Creatinine clearance < 60 cc/min
	B. Calciuria 24 h > 400 mg/d (>10 mmol/dL) and increased risk of calculosis by biochemical analysis
	C. Presence of nephrolithiasis or nephrocalcinosis by X-ray, ultrasound, or CT
4. Age	<50 years

Table 1.
Indication of parathyroidectomy in asymptomatic primary HPT [21].

Tertiary HPT occurs in autonomy of parathyroid glands resulting from continuous stimulation of PTH secretion, a consequence of secondary HPT. These cases are better demonstrated after kidney transplantation, when PTH levels are kept high even after treating the cause of secondary HPT, the renal disease. Surgical treatment is indicated due to persistent hypercalcemia, in addition to symptoms that may be associated, such as bone pain, pathological fractures, and intractable pruritus. The surgical approach, as in secondary HPT, can be total parathyroidectomy with autograft of parathyroid fragments in muscle tissue, or subtotal parathyroidectomy [36, 54]. The measurement of intraoperative rapid PTH in cases of secondary and tertiary HPT during surgical treatment is the subject of much current discussion, although it is increasingly accepted in order to avoid new approaches due to recurrent or persistent disease [36, 51, 55, 56].

8. Conclusion

Hyperparathyroidism is an important clinical condition with severe consequences if it is not well diagnosed and treated. Knowledge of calcium metabolism is essential for the correct management of the patient with the disease. Primary hyperparathyroidism, although oligosymptomatic in most patients, should be viewed with magnitude by general practitioners with a basic health approach. The patient with hyperparathyroidism secondary to chronic kidney disease, often with several associated comorbidities, needs the best possible approach, directly influencing his quality of life, improving cardiovascular and osteometabolic status.

Conflict of interest

The author declares no conflict of interest.

Author details

Andre Luis Maion Casarim
School of Medical Sciences, Universidade Estadual de Campinas, Campinas, Brazil

*Address all correspondence to: andre.casarim@yahoo.com.br

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Section 4

Parathyreoid Glands and Their Diseases

Chapter 6

Calcium and Metabolic Bone Disorders

Ayotunde Oladunni Ale, Oluwayomi Akande and David Da Rocha-Afodu

Abstract

Calcium homeostasis has a pivotal role in regulating many biological processes. The interplay of calcium-regulating hormones, including parathyroid hormone (PTH), vitamin D, and calcitonin, is crucial in tightly maintaining serum calcium levels. Deregulation of calcium homeostasis has clinical implications resulting in hypercalcemia or hypocalcemia, which can lead to metabolic bone disease (MBD). MBD is a group of multifactorial bone diseases, caused by bone demineralization and characterized by an increased susceptibility to fracture risk. This chapter aims to provide an overview of associated risk factors and diagnostic, prevention, and recent treatment methods for MBD. The diagnosis of MBD is based on the assessment of clinical signs, radiological findings, quantitative ultrasonography, and biochemical evaluation of serum calcium, phosphate, PTH, alkaline phosphatase, and vitamin D. Current pharmacological treatments include antiresorptive and anabolic conventional therapies. Additionally, the efficacy of herbal extracts and nutritional supplements have been evaluated. Recent advances in the MBD management include drugs targeting calcium-sensing receptor and parathyroid hormone-related proteins, leading to the development of cathepsin K and Src tyrosine kinase inhibitors, calcilytics, and monoclonal antibodies against sclerostin or Dickkopf-1. Moreover, new nanomaterials have been used for improving the surgical treatment of vertebral fractures.

Keywords: calcium, parathyroid hormone, metabolic bone disease, osteoporosis, vitamin D, anabolic drug, antiresorptive drugs

1. Introduction

Metabolic bone disease (MBD), the third most prevalent disorder of the endocrine system, involves any disorder that alters the phenomena of mineralization in the normal skeleton. The disorder is primarily caused by abnormalities in the structure of bone or its mass, vitamin D level as well as the presence of certain minerals such as calcium and phosphorus [1].

The concentration of extracellular calcium is crucial for several functions at the cellular level, which needs to be retained in restricted levels. The free concentration of calcium is predominantly negatively regulated by the secretion of the parathyroid hormone (PTH) in response to calcium-sensing receptors. A substantial drop

in the level of free calcium activates the release and synthesis of PTH, which often leads to calcium reabsorption in the renal tubules, enhanced secretion of calcitriol (vitamin D3) promoting calcium absorption from the intestine, and immediate release of calcium from the skeleton, which contains 99% of calcium in the body. Conversely, in regard to rising levels of calcium in the body, PTH level drops that lead to a decline in the above-stated processes. This balance is seen to be disturbed in various pathological circumstances leading to elevated or low calcium levels. High calcium levels, known as hypercalcemia, and low calcium levels, known as hypocalcemia, are observed in conditions such as hypoparathyroidism and vitamin D deficiency.

The most common forms of MBD comprises of osteoporosis, osteomalacia, primary hyperparathyroidism, and fluorosis, while fibrous dysplasia, Paget's disease, osteogenesis imperfecta, and tumor-induced osteomalacia account for its rare forms.

Osteoporosis is a severe MBD that constitutes to be a serious health issue for older people. It represents a decline in the bone mass per unit volume, leading to significant weaknesses in the bone structure, which ultimately leads to bone deformity/fracture. Osteoporosis is categorized as primary when there is no prominent diagnosis of the disease and secondary when an established contributing cause such as steroid treatment is detectable. Type I (postmenopausal) and type II (age-related) are categorized under primary osteoporosis. Type I osteoporosis incorporates bone loss with the expedited bone mass reduction due to the withdrawal of estrogens [2].

Osteomalacia results from curtailed absorption of calcium and phosphate in the intestine due to a deficiency in vitamin D or more rarely due to calcium or phosphate deficiency. Joint pain with fragility in bone and muscular weakness are the common symptoms observed in patients with osteomalacia [3].

Paget's disease leads to skeletal lesions resulting in progressive bone turnover. The finely constructed bone lacks a natural lamellar framework and has poor quality with effects like bone deformity with prominent fractures and related pain [4].

Hyperparathyroidism results due to excess secretion of PTH, which can be categorized as primary hyperparathyroidism or secondary hyperparathyroidism. Primary hyperparathyroidism occurs due to the raised concentration of calcium in the serum. Research reports show hypercalcemia with an abnormally high level of alkaline phosphatase and elevated serum PTH [5].

Fibrous dysplasia is categorized as a rare form of metabolic disorder in which the bones are covered with irregular structures, which appear as a scar-like fibrous tissue. This deposited structure affects bone structure and integrity, making it more fragile and fracture-prone.

This chapter discusses in brief about the associated risk factors and diagnosis of MBD along with the preventive measures and the pharmacological approaches for the treatment of MBD.

2. Associated risk factors

Several contributing factors that control bone mass are diet, lifestyle, levels of cytokines, level of mobilization and physical activity, hormones, genetic factors, and local growth factors. **Table 1** illustrates premature risk factors associated with MBD for both antenatal and postnatal period.

The amalgamation of various nutritional and biomechanical factors results in the precipitation of MBD. Some of them are discussed below:

Antenatal	Postnatal
Preclampsia	Liver and kidney disease
Placental insufficiency	Use of drugs such as loop diuretics, methylxanthines, glucocorticoids
Prevalence of neuromuscular disorders, intraventricular hemorrhage	Prevalence of bronchopulmonary dysplasia

Table 1.
List of premature risk factors associated with metabolic bone disorders for both antenatal and postnatal period.

2.1 Vitamin D deficiency

Vitamin D is inevitable for retaining the rate of metabolism in bone. The major function of vitamin D is to boost calcium and phosphorus intestinal absorption by its active metabolite 1,25dihydroxyvitamin D3 along with fostering the continuance of neuromuscular function as well as bone remodeling. Disorders in which this active metabolite is deficient can pose a greater risk of the incidence of bone disorders [6]. Low levels of vitamin D results in decreased absorption of intestinal calcium and phosphorus, with a drop in the level of calcium in serum with an increased synthesis of PTH. A rise in the level of PTH in plasma preserves the level of normal serum calcium by enhancing 1,25-$(OH)_2$D renal development, growing bone yield, and escalating loss in mass of bone. Lack of sufficient intake or a maternal lack of vitamin D is the most leading cause of deficiency of vitamin D. Renal failure or the incidences of hepatic disease, receptor defects, or synthesis of congenital vitamin D are the other instances that cause a vitamin D deficiency. Additionally, two other rare genetic diseases, including vitamin D-dependent rickets type 1 or pseudovitamin D deficiency rickets, are caused due to the mutation in the gene encoding 1α-hydroxylase enzyme (CYP27B1 gene), which is a rate-limiting enzyme involved in the bioactivation of vitamin D.

A recent report has evaluated vitamin D status and its relationship with skeletal health in 40 healthy adult Nigerians (aged between 21 and 50 years) [7]. An array of physiological parameters were evaluated, which predominantly included markers of bone health, thyroid function and renal function, levels of parathyroid hormone, calcium excretion rates, and serum 25-hydroxyvitamin-D levels. The observed results indicated the fact that approximately 70% of the reported cases had an incidence of vitamin D insufficiency with 25% of the subjects indicated osteopenia, while none of the subjects presented with osteoporosis. The bone mineral density (BMD) T-score for osteopenic subjects was significantly lower than for non-osteopenic subjects. It was also observed that osteocalcin levels in serum were considerably higher in osteopenic subjects versus non-osteopenic subjects; however, a 24-hour calcium excretion was comparable between the two groups. Mean serum 25-hydroxyvitamin-D was lower in subjects with osteopenia compared to non-osteopenic subjects, while parameters for thyroid, renal, and calcium-phosphorus were not significantly different in the observed group [7].

2.2 Disorders related to homeostasis of calcium and phosphorus

Disorders related to homeostasis of calcium and phosphorus results in ultimate clinical consequences for neonates. A fine positive balance between calcium and phosphorus is indispensable for sufficient bone growth and maturation. Neonates with persistent malabsorption are at high prospects of poor absorption of calcium, phosphorus, magnesium, or vitamin D, either due to medical or surgical interventions [8].

2.3 Drug-related factors

Some drugs that are frequently used in premature births also support the incidence of MBD. Some of the prominent classes of such drugs are loop diuretics such as furosemides, corticosteroids, methylxanthines, antifungals, and certain antiepileptics. The most probable reason may be activation of osteoclasts and reduction of osteoblast proliferation and decreased absorption, thereby the ultimate elimination of calcium by the kidneys [9].

2.4 Parent related nutrition

The concentration of minerals such as calcium and phosphorus in premature breast milk is inadequate in regard to the estimated requirement, presuming that they ingest approximately one third that is essential in fetal life [9]. In addition, milk products are high in concentration of the stated minerals but have a lower bioavailability; hence, consumption of mineral fortified milk is essential for preventing and treating MBD.

2.5 Biomechanical factors

Biomechanical factors that impact the alteration of bone structure is accountable for the reduction of bone mass caused by reduced activity level. The majority of bone-loading process occurs during the third trimester. Nevertheless, in the absence of bone loading, bone formation stops and further osteoclasts are activated leading to a reduction in bone strength [10]. Neonatal demineralization of the skeleton may result from immobilization due to the prevalence of other disease conditions or neurological implications.

2.6 Endocrinology-related factors

Thyroid hormones are prerequisite for the development of the skeleton and are prime regulators of bone maintenance. Hypothyroidism induces delayed development of the skeleton and growth retardation with delayed bone development owing to inadequate endochondral ossification. Hyperparathyroidism also impacts bone metabolism, which causes significant conditions such as hypercalcemia, demineralization of the bone, and delay in growth and development. Due to these abovementioned-stated issues, a decline in the normal function of kidneys eventually leads to mineral and bone metabolism disturbances culminating in serious skeletal deformities [11].

3. Diagnosis

Since there are no ultimate diagnosis and therapy indications for MBD, and the related sign and symptoms also appear very late, it is, therefore, appropriate to monitor the subjects at risk for the development of the related disorder.

3.1 Serum markers

Levels of alkaline phosphatase (ALP) rise physiologically at about 6–12 weeks of age over the first 3 weeks of life. Regardless of the lack of signs and symptoms, ALP levels > 500 IU/L suggest impaired bone homeostasis and values >700 IU/L is associated with bone demineralisation [12].

Serum phosphate levels <5.6 mg/dl are strongly linked with the prevalence of the radiologically apparent disorder in preterm infants with an average gestational age of 24.7–33.0 weeks [13].

3.2 Urinary markers

Hypophosphatemia is the most prevalent physiological modification coupled with premature MBD, which causes a reduced release of PTH and thereby increases the reabsorption of phosphate from the renal tubular. Infants born <28 weeks of gestation have a reduced baseline value for phosphate, resulting in increased excretion of phosphate in urine, even in the mere existence of lower levels of phosphate that appear as a significant marker for MBD incidence [14].

3.3 Radiological markers

Dual-energy X-ray absorptiometry (DEXA) is the conventional method used for BMD assessments. DEXA employs the use of low ionizing radiation and measures the calcium content in bone in terms of grams of hydroxyapatite/cm^2.

Quantitative ultrasound is another technique that is relatively inexpensive and measures the mineral content of bone as well as the organic matrix. The parameters that are evaluated by the abovementioned technique are the speed of sound and bone transmission time [15].

4. Prevention of MBD

There are certain non-pharmacological approaches that need to be inculcated in daily life for the prevention of MBD. Some of them are discussed below:

4.1 Physical activity

Individuals with MBD should be educated about the potential advantages of physical activity and motivated to be active within their ability and in keeping their values and goals as realistically possible. They should be given training on how to self-monitor for signs and symptoms that should be brought to their healthcare team's attention and the emergency contact information for this team should be issued [16].

4.2 Adequate calcium and vitamin D intake

The Institute of Medicine (IOM) prescribes that dietary calcium consumption should be limited to 1000 mg daily for men aged 50–70 years, and 1200 mg daily for women aged 51 years and over [17]. Presently, the impact of calcium supplementation on stone formation is unclear. Large doses of supplemental calcium are likely to lead to stone formation, especially if given separately from a meal. If appropriate, patients with stones should be advised to take a meal with calcium supplements, and further, the disease condition needs to be closely monitored [18].

Vitamin D is a vital component of calcium absorption, which helps in the maintenance of bone health. The IOM recommends 600 IU and 800 IU per day for men and women who are aged 51–70 years and over 70 years, respectively [17]. Earlier reports indicate the fact that combined vitamin D and calcium intake demonstrated a reduction in the risk of fracture in older adults, but the effects varied according to

the study setting, i.e., institution versus community dwellers. The risk of fracture among older adults was lower in the community dwellers than for institutionalized elderly people. However, further research is required for appropriate dose and dosing regimens to end up in a conclusive remark [19].

4.3 Adequate protein intake

Maintaining an appropriate intake of proteins is vital for maintaining musculoskeletal functioning in postmenopausal women and men over the age of 50 years. The recommended protein intake is 0.8 g/kg/day [20].

4.4 Reducing the intake of caffeine

The impact of various caffeinated beverages has been inferred as a trigger of osteoporosis and fragility fracture in individuals; hence, it is recommended to restrict the intake of caffeine [21].

5. Treatment approaches for MBD

The recent decade has witnessed much progress in the introduction of new medications for the treatment of MBD. The treatment modality of this group of disorders comprises two major treatment regimens, antiresorptive and anabolic conventional therapies. Antiresorptive drugs predominantly reduce the bone resorption rate, while anabolic drugs boost the formation of bone. The following medicines for skeletal disorders, including Paget's disease of the bone, osteoporosis, MBD, and several other rare type of bone diseases, form the basis of our current clinical treatment regimen.

5.1 Antiresorptive agents

The major class of drugs included in this category includes bisphosphonates, estrogens, calcitonin, and denosumab.

Bisphosphonates, first-line antiresorptive bone agents, are commonly used to treat osteoporosis caused by glucocorticoids and other disorders marked by severe osteoclastic bone resorption, such as humoral malignant hypercalcemia, Paget's disease, multiple myeloma, and osteolytic bone metastasis [22]. The drugs specifically included in this group for the treatment of MBD comprises of alendronate, risedronate, and zoledronic acid. Such groups of therapeutic agents bind with a high affinity to the bone's mineral matrix and prevent resorption of osteoclast of the bone, resulting in reduced bone turnover and a significant increase in bone mass [23]. The most prominent side effect related to bisphosphonates administered orally is the upper gastrointestinal discomfort, which majorly includes the erosion of the esophagus leading to ulcer, heartburn, and indigestion.

Calcitonin is approved for the treatment of osteoporosis care in postmenopausal women when alternative therapies are not practicable [24].

Denosumab, the first biological agent available for osteoporosis treatment, is a fully human monoclonal antibody that acts by inhibiting transmembrane protein (RANKL), which has proven results for the formation and functioning of osteoclasts, thereby reducing bone resorption. It is usually recommended for the patients who are unable to be on drug therapy, which are orally administered

but are at high risk for the incidence of fractures. Denosumab is well-tolerated, but associated hypersensitivity or dermatological reactions, musculoskeletal pain, infections, and hypercholesterolemia are the major documented adverse effects. It can trigger hypocalcemia, so calcium levels should be fixed before starting treatment [25, 26].

5.2 Estrogen agonist/antagonist

Estrogen therapy is FDA approved exclusively for the prevention of osteoporosis in postmenopausal women who are at high risk, and should only be used when non-estrogenic osteoporotic medications have been deemed inappropriate. Hormonal replacement therapy is no longer recommended as a first choice for treating and preventing osteoporosis in postmenopausal and premenopausal women due to the overall associated health risks that hugely outweigh the benefits.

While antiresorptive drugs usually display a lower incidence of associated side effects, bone turnover suppression can elucidate the necrosis of the jaw and the incidence of atypical femur fractures that can be documented in patients with long-term bisphosphonate usage [27]. Because antiresorptive agents are unable to preserve bone mass and bone integrity, it continues to be of core interest to identify molecular targets that would promote osteoblast activity and lead to enhanced bone mass with reconstructed skeletal architectures.

5.3 Anabolic conventional therapies

Osteoanabolics are another category of drugs, which covers the PTH and parathyroid hormone-related peptide analogs. PTH functions as an efficient endocrine regulator for the maintenance of calcium and phosphate concentrations in extracellular space, vital to the preservation of concentration of calcium in serum and urinary samples within the normal physiological limit. High PTH levels lead to a high bone-turning state with bone resorption exceeding bone formation and ultimately osteoporosis precipitation [28].

Teriparatide was the first anabolic treatment option approved for the treatment of osteoporosis, which has a mode of action similar to that of the PTH hormone. This works by triggering the development of new bone by increasing osteoblastic development when given in low doses [29]. In patients with Paget's bone disease, elevated concentrations of alkaline phosphatase, prior skeletal radiotherapy, recurrent or metastatic bone malignancy, hypercalcemic disorders such as primary hyperparathyroidism, avoidance of the treatment is suggested [30]. Abaloparatide is another FDA approved drug for the treatment of osteoporosis in postmenopausal women. It is further advised to avoid the treatment in patients with preexisting hypercalcemia and disorder such as primary hyperparathyroidism [31].

Another promising investigational drug is romosozumab, which is a sclerostin-neutralizing antibody. Reports have shown elsewhere that it is better alternative bisphosphonate alendronate in women with severe osteoporosis for reducing the risk of prominent clinical fractures. This was accompanied by a boost in bone formation markers with a decline in bone resorption markers, implying the action of both stimulating bone formation and inhibiting bone resorption [32].

Apart from these two major classes of drugs, various herbal medicines are also gaining attention for being used in the treatment of MBD. Some of them include Hachimi-jio-gan and Juzen-taiho-to, *Kami-kihi-to*, *Bushenningxin*, *Shu Di Shan Zha*,

Class of drug	Investigational drug	Characteristics	Mode of action	Therapeutic efficacy	References
Calcilytics	MK-5442 (Phase II)	Orally bioavailable	CaSR antagonist	Transient PTH pulses and a dramatic rise in the formation of bone markers were noted, with a transitory significant decline in markers of bone resorption. Compared to placebo, no further rise in BMD was reported	[36]
Cathepsin K inhibitors	ODN	Long half-life, orally bioavailable	Inhibits CatK from binding to its corresponding substrates	Reduced bone turnover in ovariectomized animals and promoted periosteal bone formation was observed	[37]
	ONO-5334	Synthetic derivative, low molecular weight, oral formulation	Inhibits CatK from binding to its corresponding substrates	In Phase 2 clinical trial in postmenopausal women with osteoporosis, there was a substantial enhancement in BMD in the lumbar spine, total hip, and femoral neck compared to placebo. The observed effect on BMD of ONO-5334 was found to have a similar effect as that of alendronate, when administered at a dose of 70 mg once weekly	[38]
Src tyrosine kinase inhibitors	Saracatinib (AZD0530)	Oral formulation	Inhibits the enzyme Src kinase competitively	A notable decrease in bone resorption markers without any noticeable effect on bone formation markers and no serious adverse effects was documented, demonstrating a reduction in osteoclast bone resorption effect of saracatinib	[39]
Monoclonal antibodies against sclerostin/ Dickkopf-1	Romosozumab (AMG-785)	Human monoclonal anti-sclerostin antibody	Monoclonal antibody against sclerostin	A rise in dose-dependent BMD at the lumbar spine and total hip with a decrease in bone resorption markers with marked improvement in bone formation markers after a period of 3 months was reported	[40]

PTH, parathyroid hormone; CaSR, calcium sensing receptor; BMD, bone mineral density; CatK, cathepsin K.

Table 2.
Recent advances for the treatment of metabolic bone disease.

and so on, which have proven reported results in various animal models on improving bone health [33, 34]. Reports suggest the fact that Hachimi-jio-gan and Juzen-taiho-to significantly prevented the loss of bone in SAMP6, a murine model for senile osteoporosis [34]. The decoction containing Bushenningxin caused osteoblasts to have an increase in the number of cell organelles with clear Golgi apparatus, increased proliferation, and inhibition of apoptosis for a time period of 12 weeks when given to OVX mice [35].

Recent advances in MBD treatment include medications that target calcium-sensing receptors and proteins linked to the hormone parathyroid, leading to the design of cathepsin K and Src tyrosine kinase inhibitors, calcilytics, and monoclonal antibodies against sclerostin or Dickkopf-1 (**Table 2**).

Nanoenabled systems for the systemic delivery of drugs for the treatment of MBD have attracted huge attention in recent times. A number of formulations were designed for the controlled delivery of medicaments for better therapeutic efficacy with minimal associated adverse effects. Some of the formulations reported in this specified category include tigecycline entrapped calcium phosphate/poly-DL-lactide-co-glycolide nanoparticles, titanium implants coated with bisphosphonate encased calcium phosphate nanoparticle, and gold nanoparticles incorporated gelatin-based hydrogel. Reports suggest that surface reconfiguration through nanotechnology has played a significant role in the design and manufacture of better spinal implants [41–44].

6. Conclusion

The burgeoning of the incidences of MBD is raising concern worldwide. Proper screening of the disorder is of prime importance in dealing with it. Although bisphosphonates remain the first-line treatment choice for the stated disorder, researchers should work upon the novel drugs with a unique mode of action and appreciable long-term safety profile. Based on the literature, it is pertinent to state that a fine balance between the non-pharmacological and pharmacological approaches could help out in dealing with MBD judiciously resulting in its prevention. Therefore, the battle for the search of better drugs for treating patients with metabolic bone diseases continues with an aim to provide better therapeutic efficacy and patient compliance.

Author details

Ayotunde Oladunni Ale[1*], Oluwayomi Akande[2] and David Da Rocha-Afodu[3]

1 Department of Medicine, Obafemi Awolowo College of Health Sciences, Faculty of Clinical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria

2 Internal Medicine, Department of Hospital Medicine, Redmond Regional Medical Center, HCA Healthcare, Rome, GA, USA

3 Nephrology and Hypertension, Renal Services of Toledo, Oregon, Ohio, USA

*Address all correspondence to: ayoale2004@yahoo.com

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Chapter 7

Severe Hypocalcemia after Total Parathyroidectomy Plus Autotransplantation for Secondary Hyperthyroidism-Risk Factors and a Clinical Algorithm

Fong-Fu Chou and Jin-Bor Chen

Abstract

Severe hypocalcemia is a serious complication occurring after parathyroidectomy for secondary hyperparathyroidism. Totally, 322 patients who were successfully treated with total parathyroidectomy and bilateral thymectomy plus autotransplantation were studied. Group A (247 patients) developed mild hypocalcemia. Group B (75 patients) who had post-operative serum Ca levels <6.5 mg/dL or needed >4 g of intravenous (i.v.) Ca gluconate to keep Ca levels ≥6.5 mg/dL developed severe hypocalcemia. Preoperatively, patient age was recorded, and serum Ca, P, alkaline phosphatase (Alk-ptase), and intact parathyroid hormone (iPTH) levels were checked. These serum levels were checked again 18 h post-operatively. The algorithm showed that i.v. Ca gluconate 8 g/150 dL (5% glucose)/day was administered for Ca levels <6.5 mg/dL, 4–6 g/75 dL/day for levels <7.6 mg/dL, and 2 g/15 dL/15 min for symptomatic hypocalcemia. Young age, low Ca, and high Alk-ptase levels and long operation time were independent risk factors for severe hypocalcemia. Serum Ca levels <7.6 mg/dL at 18 h post-operation were the optimal cutoff value for hypocalcemia that needed i.v. Ca gluconate. The post-operative hospitalization in Group B was 3–5 days shorter than that previously reported. The readmission rate (0.62%) due to hypocalcemia was rare.

Keywords: secondary hyperparathyroidism, total parathyroidectomy plus autotransplantation, severe hypocalcemia, intravenous calcium gluconate

1. Introduction

In patients with end-stage renal disease (ESRD), parathyroid hyperplasia, high circulation parathyroid hormone (PTH), and hyperphosphatemia characterize secondary hyperparathyroidism (SHPT).

SHPT is a serious manifestation of chronic kidney disease (CKD) with negative effects on patients' life quality and outcome.

In ESRD, medical treatment for secondary hyperparathyroidism has three main strategies: reduction of P uptake by dialysis, dietary restriction, and/or P-binders; calcimimetics; and vitamin D.

Due to toxicity, aluminum-based P-binders have been replaced by those containing Ca salts. At high doses, Ca-based P-binders may elevate the risk of vascular calcification. Ca-free P-binders with dietary P restriction appear to lower fibroblast growth factor-23 and improve cardiovascular and renal outcomes in patients with SHPT [1].

Despite the availability of several P-binders, the ideal P-binder that combines high efficacy, low pill burden, minimal side effects (including gastro-intestine), and low cost is still not available [2], and the effect on survival is unclear [3].

In EDRD patients, vitamin D may improve abnormal mineral homeostasis; however, a steady escalation of vitamin D analog dose is not feasible due to hypercalcemia, hyperphosphatemia, and/or parathyroid gland resistance, despite the concurrent use of calcimimetics [4].

Calcimimetics such as cinacalcet therapy are currently a class of agents that activate the Ca sensing receptor and potentiate the effect of extracellular Ca. Literature supports cinacalcet therapy to improve patients' outcomes, especially with regard to vascular calcifications and presumably the very lethal condition of calciphylaxis [5].

Additional clinical evidence suggests that cinacalcet in combination with low-dose vitamin D is more effective in lower PTH than calcitriol alone. However, cinacalcet is administered orally and has been associated with gastrointestinal intolerance along with hypocalcemia [6].

In addition, poor adherence has been observed among dialysis patients self-administering cinacalcet [5]. Cost effectiveness is another consideration; the addition of cinacalcet contracts an additional US$3000–4000 per year on the top of the costs of vitamin D and P-binders [7].

If calcimimetics side effects are intolerable, some researchers have reported that parathyroidectomy may be more cost-effective than cinacalcet in some patients with ESRD and suffering from uncontrolled SHPT [4].

2. Parathyroidectomy rates, indications, and methods

Parathyroidectomy was required in about 10% of patients after 10 years and 20% after 20 years in dialysis patients [8]. The parathyroidectomy rate was 8.8/1000 patient-years from 1991 to 2009 in the Swedish dialysis and transplant population [9]. A trend toward a dip in parathyroidectomy rate was found during the era of cinacalcet. This change in treatment strategy was accompanied with increased preoperative PTH levels reflecting delayed surgery and increased disease severity [10].

The overall rate of parathyroidectomy in the United States was approximately 5.4/1000 patients between 2002 and 2011. The rate decreased from 2003 (7.9/1000 patients), reached a nadir in 2005 (3.3/1000 patients), increased again through 2006 (5.4/1000 patients), and remained stable since that time. Rates of in-hospital mortality after parathyroidectomy decreased from 1.9% in 2003 to 0.8% in 2011 [11].

In-hospital mortality has seldom happened in Kaohsiung Chang Gung Memorial Hospital during 30 years in over 2000 patients undergoing parathyroidectomy plus autotransplantation for secondary hyperparathyroidism, owing to routine cardiac 2D-echography, thallium-201 myocardial imaging, and EKG examinations before surgery [12]. In recent 5 years, sestamibi parathyroid scintigraphy is also routinely performed preoperatively.

The indications for parathyroidectomy are symptoms of bone pain, skin itching, general weakness, insomnia, and soft tissue calcification with Ca levels ≥10.2 mg/dL, P levels ≥4.7 mg/dL, alkaline phosphatase (Alk-ptase) levels ≥94 IU/L, intact parathyroid hormone (iPTH) levels ≥800 pg./mL, and bone mineral density (T-score) ≤ −2.5

in dialysis patients. All oral medications including calcitriol, sevelamer, and cinacalcet have to be discontinued 1 month before surgery to avoid severe hypocalcemia in the post-operation period.

During surgery, if four or more glands and bilateral thymus are removed (total parathyroidectomy and bilateral thymectomy) (TPX & BT), l00 mg of parathyroid gland with diffuse hyperplasia is autotransplanted (AT) into the subcutaneous tissue of the forearm without harboring the arteriovenous fistula [13]. If less than four glands are found and removed, bilateral thymectomy is performed, but AT is omitted.

3. Definition of severe hypocalcemia post-parathyroidectomy and risk factors

Previously, the critical value of hypocalcemia (CVH) was defined as Ca levels ≤6.0 mg/dL within 48 h of total parathyroidectomy, indicating the possibility of life threatening complications [14] or as profound and prolong hypocalcemia (hungry bone syndrome) with corrected serum Ca levels of ≤8.4 mg/dL lasting for 4 or more days, that occurred anytime within 1 month following the parathyroidectomy [15]. Bone hungry syndrome occurred frequently around 25–27.4% after total parathyroidectomy for secondary hyperparathyroidism [4, 15] and CVH around 15.3% [14].

In a recent study, we included 322 patients who were successfully treated with TPX & BT plus AT. They were divided into two groups. Group A (mild hypocalcemia) patients had serum Ca levels ≥6.5 mg/dL at 18 h post-operation and needed ≤4 g i.v. Ca gluconate to keep Ca levels ≥6.5 mg/dL during the post-operative period (7 days). Group B (severe hypocalcemia) patients had serum Ca levels <6.5 mg/dL at 18 h post-operation or needed >4 g of i.v. Ca gluconate during the post-operative period to keep Ca levels ≥6.5 mg/dL. Surgery was considered successful when iPTH levels were lowered to <72 pg./mL within 1 week after surgery [16]. The rate of severe hypocalcemia was 23.3% in our study. It appeared that our study included a larger sample size than previous series did [14, 15, 17–20]; thus, our results were more dependable, but a few risk factors we identified were different from those reported previously.

Using the ROC curve analysis of Ca levels at 18 h post-operation for predicting hypocalcemia that needed i.v. Ca gluconate, the maximal Youden index was 0.415 and the optimal cutoff value was 7.6 mg/dL, with sensitivity of 0.72 (95% CI 0.590–0.839), specificity of 0.695 (95% CI 0.620–0.748), and area under the curve 0.749 ± 0.032 (mean ± SE) (95% CI 0.686–0.812) (**Figure 1**); patients who met this criterion should be treated with i.v. Ca gluconate. Previous reports suggested that Ca levels of 7.5–8.0 mg/dL at 18 h post-operation could predict severe hypocalcemia, and our results support this finding [18, 20].

Preoperatively, patients were younger in Group B [50 (40–46)] [median (interquartile range)] than in Group A [58 (52–64)] ($p < 0.001$); serum P, Alk-ptase, and iPTH levels were significantly higher, but serum Ca levels were significantly lower in Group B than those in Group A (**Table 1**). Same findings were reported previously [14, 15]. There were no significant differences between the two groups in terms of sex, symptoms, body weight, and duration of dialysis (**Table 1**). The amount of blood loss during surgery was not significantly different between the two groups. The operation time, total weight of removed parathyroid glands, duration of post-operative hospitalization (days), and total amount of i.v. Ca gluconate administered were significantly more, but calcium levels at 18 h post-operation were significantly lower in Group B than in Group A ($p = 0.014$, $p = 0.035$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively) (**Table 2**).

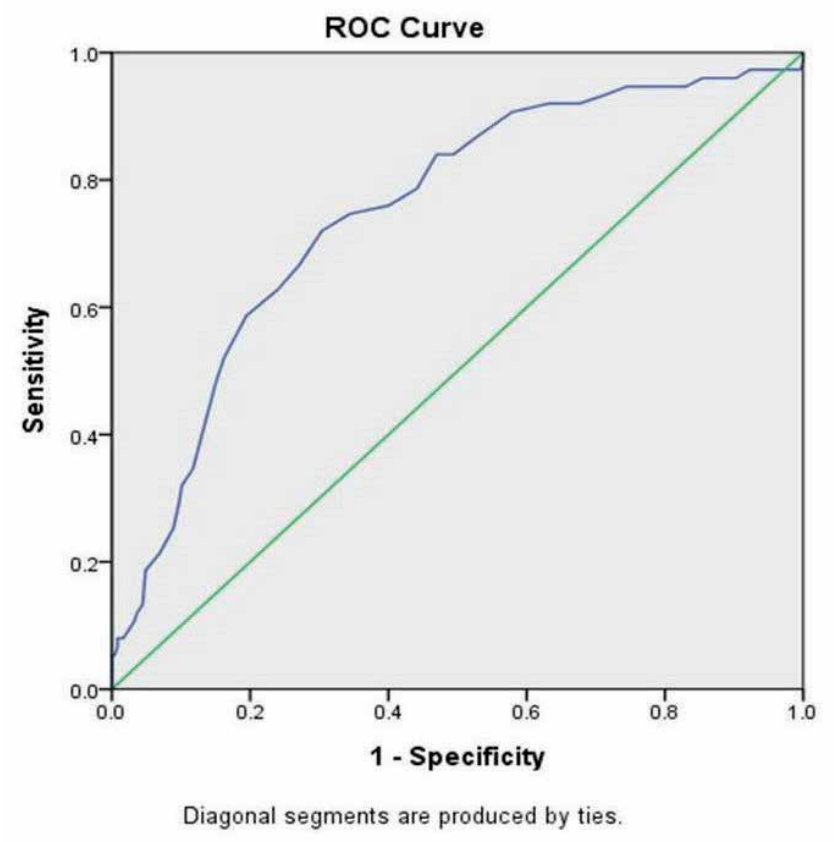

Figure 1.
Using receiver operating characteristics (ROC) curve, maximal Youden's index = 0.415, sensitivity = 0.72, 1-specificity = 0.305, area under curve = 0.749 ± 0.032 (SE), 95% confidence interval = 0.686–0.812, and optimal cutoff calcium value = 7.6 mg/dL (at 18 h post-operation) to predict severe hypocalcemia.

We believe that younger patients have better bone-formation abilities than older patients; thus, they are more likely to have severe hypocalcemia after parathyroidectomy [15, 21, 22].

In our study, severe hypocalcemia occurred in 75 patients (23.3%), which is lower than the rates (27.4–97%) reported previously, likely owing to the definition of severe hypocalcemia [15, 20–22].

We found that mean preoperative Ca levels were lower in Group B than in Group A but Alk-ptase levels in Group B were higher than in Group A (**Table 3**). The cause was not very clear, but this had also been reported previously [15, 20–22].

Before patient discharge, Ca levels in Group B were lower than in Group A, but Alk-ptase levels in Group B were higher than in Group A (**Table 3**). Although it was unclear whether serum Ca levels in patients with severe hypocalcemia remained significantly lower throughout the year after operation, as reported previously [15], we found that all patients in our study could maintain Ca levels >8.0 mg/dL after 3 months with the use of oral Ca carbonate <3 g/day and calcitriol <0.5 μg/day. We speculated that the autotransplanted parathyroid tissue might start to function 1–3 weeks later, as reported previously [23].

Preoperative P levels were higher in Group B than in Group A, which had rarely been reported previously [24].

High Alk-ptase levels are characteristic of bone diseases involving increased osteoblastic activity, bone formation, and resorption in secondary

[Normal ranges]	Group A (N=247) Mild hypocalcemia	Group B (N=75) Severe hypocalcemia	p
Age	58 (52–64)	50 (40–64)	<0.001
Sex M/F	96/151	37/38	0.111*
Skin itching (+/-)	161/86	54/21	0.328*
Bone pain (+/-)	176/71	53/22	1.0*
General weakness (+/-)	121/126	32/43	0.358*
Insomnia (+/-)	154/93	44/31	0.590*
Body weight (kg)	57.1 (49.9–64.9)	59.2 (52.5–69.9)	0.122
Duration of hemodialysis (years)	10 (6.8–13) (N=207)	8.0 (5.8–11.3) (N=58)	0.205
Duration of peritoneal dialysis (years)	6.0 (4.3–8.8) (N=40)	7.0 (5.0-9.5) (N=17)	0.629
Ca [7.9-9.9 mg/dL]	10.4 (10–10.9)	10.3 (9.5–10.7)	0.007
P [2.4-4.7 mg/dL]	5.6 (4.6–6.7)	6.4 (5.7–7.1)	<0.001
Alk-ptase [28-94 IU/L]	141 (99–228)	227 (169–420)	<0.001
iPTH [14-72 pg/mL]	1298 (1025–1750)	1740 (1295–2359)	<0.001

** Using X^2-test.*
Using Mann-Whitney U test.
All data = median (interquarter range) (IQR).
Alk-tase = alkaline phostaphatase.
iPTH = intact parathyroid hormone.

Table 1.
Age, sex, symptoms, body weight, duration of dialysis and preoperative serum calcium (Ca), phosphorus (P), Alkaline-phosphatase (Alk-ptase) and intact parathyroid hormone (iPTH) levels. Comparison between mild hypocalcemia (Group A) and severe (Group B) hypocalcemia groups.

	Group A (N=247) Mild hypocalcemia	Group B (N=75) Severe hypocalcemia	p
Blood loss (cc)	15 (10–20)	17.5 (10–21)	0.560
Operation time (min)	131 (117–156)	145 (122–166)	0.014
Total weight of removed parathyroid glands (g)	3.3 (2.3–4.8)	3.8 (2.8–5.4)	0.035
Days of postoperative hospitalization	5 (5–5) 5.0 ± 1.1	5 (5–8) 6.4 ± 2.5	<0.001 <0.001*
Total i.v. Ca gluconate (g)	0 (0–0)	12 (8–18)	<0.001
Ca levels 18 h after operation	8.2 (7.5–8.8)	7.2 (6.6–7.8)	<0.001

** Using Student't test.*
Using Mann-Whitney U test.
Data = median (interquarter range) (IQR).
Data = mean±SD (standard deviation).

Table 2.
Blood loss during surgery, operation time, total weight of removed parathyroid glands, days of postoperative hospitalization and total intravenous (i.v.) Ca gluconate and Ca levels at 18 h post-operation. Comparison between mild (Group A) and severe (Group B) hypocalcemia groups.

hyperparathyroidism. Preoperative Alk-ptase levels were higher in Group B than in Group A. We found that preoperative Alk-ptase levels were an independent risk factor for severe hypocalcemia, similar to previous reports [14, 15, 17–19, 24].

After TPX & BT plus AT, Alk-ptase levels increased progressively, reflecting increased bone formation, which peaked at 2 weeks [15] and decreased gradually

[Normal ranges]	Group A (N = 247) Mild hypocalcemia	Group B (N = 75) Severe hypocalcemia	p
Ca [7.9–9.9 mg/dL]	7.3 (6.8–7.9)	7.0 (6.6–7.7)	0.013
P [2.4–4.7 mg/dL]	4.0 (3.2–5.1)	4.1 (3.2–4.9)	0.958
Alk-ptase [28–94 IU/L]	192 (113–343)	349 (191–636)	<0.001
iPTH [14–72 ng/mL]	8.8 (2.9–16.4)	10.1 (2.8–20.6)	0.343

Using Mann-Whitney U test.
All data = median (interquarter range) (IQR).

Table 3.
Serum calcium (Ca), phosphate (P), alkaline phosphatase (Alk-ptase) and intact parathyroid hormone (iPTH) levels at the day of discharge. Comparison between mild (Group A) and severe (Group B) hypocalcemia groups.

Risk factors unit	Coefficient	p	Odds ratio	95% CI
Age 1year	−0.045	=0.001	0.956	0.931–0.982
Ca 1 mg/dL	−0.520	0.007	0.595	0.409–0.866
Alk-ptase 1 IU/L	0.003	<0.001	1.003	1.001–1.004
Operation time 1 min	0.010	0.016	1.010	1.002–1.018

Using binary logistic regression test.
Ca = calcium levels.
Alk-ptase = alkaline phosphatase.

Table 4.
Risk factors of severe hypocalcemia after total parathyroidectomy plus auto transplantation.

to normal levels at 3 months post-operation (**Table 4**). Before patients were discharged in our series, the mean Alk-ptase level was still higher in Group B than in Group A showing that oral Ca carbonate and calcitriol should be continually administered for 2 weeks to 3 months, according to serum Ca levels [15].

Preoperatively, high iPTH levels were a clear indicator of the severity of renal hyperparathyroidism and bone disease. Preoperatively, high iPTH levels increased both bone formation and bone resorption; after parathyroidectomy, bone resorption would decrease and bone formation would increase; thus, severe hypocalcemia could develop after surgery [14, 22, 24].

We found that the total weight of the removed parathyroid glands was more in Group B than in Group A, as was the operation time. The total weight of parathyroid glands and the operation time contributed to severe hypocalcemia, might be due to advance disease and extensive dissection during surgery, but were rarely reported before [20, 25, 26].

Multi-variant binary logistic regression test showed that young age, low preoperative Ca levels, high preoperative Alk-ptase levels, and long operation time were independent risk factors for severe hypocalcemia, with associated odds ratio of 0.956, 0.595, 1.003, and 1.010, respectively ($p = 0.001$, $p = 0.007$, $p < 0.001$, and $p = 0.016$, respectively) (**Table 4**).

In our study, Ca levels were negatively correlated with P levels ($r = -0.255$, $p < 0.001$), and Alk-ptase levels were positively correlated with iPTH levels ($r = 0.449$, $p < 0.001$); therefore, preoperative Ca and Alk-ptase levels were finally identified as independent risk factors for severe hypocalcemia combined with young age and long operation time.

4. Treatment of severe hypocalcemia with our algorithm

Cozzolino et al. [27, 28] proposed a dose corresponding to the rate of 1–2 mg/kg/h for i.v. Ca gluconate, which could be increased or decreased by 25–50% from the initial value. Loke et al. [17] developed a titration regimen in which a 10% Ca gluconate infusion was started at 4.5 mL/h when serum Ca levels were < 8 mg/dL and then increased to 6.5 mL/h and finally to 9.5 mL/h if Ca levels continually declined. The algorithms they proposed were too complicated for clinical applications, and therefore, we modified it into our clinical algorithm (**Figure 2**). We adopted the clinical algorithm developed by Cozzolino et al. [28], with some modifications.

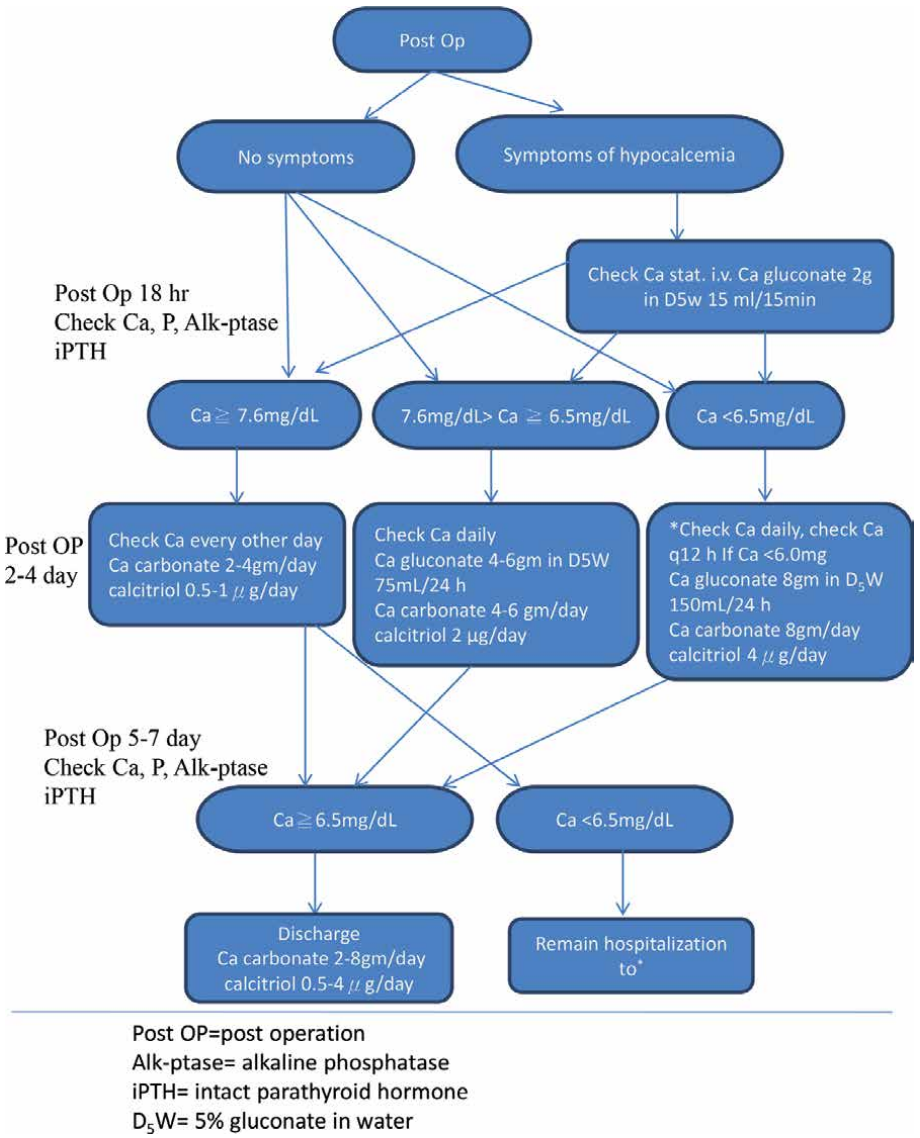

Figure 2.
The clinical algorithm for the treatment of hypocalcemia after total parathyroidectomy plus autotransplantation for secondary hyperparathyroidism.

At 18 h post-operation, Ca, P, and iPTH levels were checked to ensure that the operation had been successful and the levels of iPTH were < 72 pg/mL and P levels were above the normal lower limit (2.4 mg/dL).

If serum Ca levels were > 7.6 mg/dL at 18 h post-operation, oral Ca carbonate 2–4 g/day and calcitriol 0.5–1 μg/day were administered; Ca levels were checked on alternate days.

If serum Ca levels were ≤7.6 mg/dL and >6.5 mg/dL at 18 h post-operation, i.v. Ca gluconate (10%) 4–6 g in 75 mL of 5% glucose in water (D5W) or normal saline was administered for 24 h, concomitant with oral Ca carbonate 4–6 g/day and calcitriol 2 μg/day; Ca levels were checked daily.

If serum Ca levels were ≤6.5 mg/dL at 18 h post-operation, i.v. Ca gluconate 8 g in 150 mL D5W was administered for 24 h, concomitant with oral Ca carbonate 6–8 g/day and calcitriol 4 μg/day; Ca levels were checked daily except Ca levels <6.0 mg/dL. In that situation, Ca levels were checked every 12 h until they reached levels ≥6.0 mg/dL.

If patients had symptoms and signs of hypocalcemia, such as paresthesia of the mouth and extremities, muscle spasms, Chvostek's sign, Trousseau's sign, seizure, tetany, EKG abnormalities, arrhythmia, and hypotension, Ca levels were checked immediately and i.v. Ca gluconate 2 g in 15 mL D5W was administered in 15 min; Ca levels were then checked as usual and treated accordingly (**Figure 2**).

At 5–7 days post-operation, if patients' Ca levels were > 7.6 mg/dL, they were discharged with oral calcium carbonate 2–4 g/day and calcitriol 0.5–2 μg/day.

If Ca levels were stable and ≥ 6.5 mg/dL, they were discharged with oral Ca carbonate 6–8 g/day and calcitriol 4 μg/day. If Ca levels were < 6.5 mg/dL, they were kept in hospitalization and treated as per the clinical algorithm (**Figure 2**).

Following the clinical algorithm post-operatively, we administrate i.v. Ca gluconate 4–6 g in 75 mL D5W or normal saline in 24 h for patients with Ca levels <7.6 mg/dL, Ca gluconate 8 g/day in 150 mL D5W or normal saline in 24 h for patients with Ca levels <6.5 mg/dL, and i.v. Ca gluconate 2 g/15 mL D5W or normal saline in 15 min for patients with symptoms and signs of hypocalcemia. More concentrated solution for continuous infusion should be infused via central line [29]. Either 10% Ca gluconate (40 mg of elemental calcium per 10 mL) or 10% Ca chloride (270 mg of elemental calcium per 10 mL) can be used to prepare the infusion solution. Ca gluconate is preferred because it causes less tissue necrosis if extravasated [30]. The amount of i.v. Ca gluconate is adjusted by serum Ca levels and duration (days), neither by patients' body weight nor i.v. speed.

The K/DOQI guidelines and others suggest that serum Ca levels should be measured every 4–6 h post-operation [27, 31], but according to our experience and some other authors [28], this is not necessary except when Ca levels are <6.0 mg/dL. In such cases, we measured Ca levels every 12 h, until they were stable and reached ≥6.0 mg/dL.

5. Duration of hospital stay

In our study, the duration of post-operative hospital stay of Group A (5.0 ± 1.1 days) (mean ± SD) was significantly shorter than that of Group B (6.4 ± 2.5 days, $p < 0.001$). The duration of post-operative stay in our series was shorter than that reported previously: 7.8 ± 2.9 days (mild hypocalcemia) versus 9.3 ± 3.9 days (severe hypocalcemia) according to Yang et al. [14] and 10.2 ± 2.3 days (mild hypocalcemia) versus 15.6 ± 6.6 days (severe hypocalcemia) according to Ho et al. [15]. It was obvious that the duration of post-operative stay in our series was 3–5 days shorter than that from previous reports of severe hypocalcemia, suggesting that the clinical algorithm we adopted was acceptable.

Causes	Mild hypocalcemia (Post-Op days)	Severe hypocalcemia (Post-Op days)
Pancreatitis	1 (24)	
Bloody stool		1 (27)
Sepsis		1 (19)
Brain infarction	1 (30)	
Hypocalcemia [gastritis]		1 (3)
[Combined with heart failure]		1 (2)
Cellulitis [lower extremity]		1 (4)

No surgical mortality.
3 months after parathyroidectomy, no one needs calcium carbonate more than 2 g/daily or calcitriol more than 0.5 μg/day to keep calcium levels over 8.0 mg/dL.

Table 5.
Causes of readmission within one month post operation (Post-Op).

6. Readmission rate

A total of 2756 parathyroidectomy procedures were performed in patients with CKD, with unplanned readmission rate of 17.2 and 6.8% due to hypocalcemia/hungry bone syndrome. In one study, readmission occurred within 30 days after discharge, but readmission for severe hypocalcemia peaked within just 10 days and decreased thereafter [32].

Post-parathyroidectomy readmission rates for patients with CKD are five times higher than those for general population [32]. Using routing AT in our series, we found that seven patients (2.1%) underwent readmission due to various causes, and only two of them were due to hypocalcemia. One patient was readmitted 3 days after discharge due to gastritis, and the other at 2 days due to hypocalcemia and heart failure. However, no mortality was observed in our series (**Table 5**).

7. Conclusions

After successful TPX & BT plus AT for secondary hyperparathyroidism, severe hypocalcemia occurred in 23.3% of patients in our series. The risk factors for severe hypocalcemia were young age, low preoperative Ca levels, high preoperative Alk-ptase levels, and long operation time. Serum Ca levels <7.6 mg/dL at 18 h post-operation were the optimal cutoff value for hypocalcemia that needed i.v. Ca gluconate. When the suggested clinical algorithm was followed, the mean duration of post-operative hospital stay due to severe hypocalcemia was short (6.4 ± 2.5 days) and readmission rate (0.62%) due to hypocalcemia was quite low.

Author details

Fong-Fu Chou[1*] and Jin-Bor Chen[2*]

1 Department of Surgery, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan

2 Department of Nephrology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung City, Taiwan

*Address all correspondence to: choulu@ms4.hinet.net and chenjb1019@gmail.com

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Chapter 8

Familial Syndromes of Primary Hyperparathyroidism

William F. Simonds

Abstract

Regulation of serum calcium in vertebrates is maintained by the actions of the parathyroid glands working in concert with vitamin D and critical target tissues that include the renal tubules, the small intestine, and bone cells. The parathyroid glands release parathyroid hormone (PTH) into the systemic circulation as is required in order to maintain the serum calcium concentration within a narrow physiologic range. Excessive secretion of PTH from one or more abnormal parathyroid glands however results in primary hyperparathyroidism (HPT), a metabolic disease typically associated with abnormally elevated serum calcium. Although HPT is typically a sporadic disease, it can represent a manifestation of an inherited syndrome. Many sporadic parathyroid tumors result from inactivating mutations in tumor suppressor genes that were first discovered by the analysis of genomic DNA from patients with HPT as part of an inherited syndrome. Somatic and inherited alterations in DNA encoding proto-oncogenes can also cause parathyroid neoplasia. Two promising future approaches for the discovery of novel genes pertinent to parathyroid tumor development are the analysis of acquired genetic alterations in DNA isolated from parathyroid tumors and the investigation of familial HPT in kindreds lacking germline mutation in the known genes predisposing to HPT.

Keywords: multiple endocrine neoplasia, MEN1, MEN2A, jaw tumor syndrome, CDC73, HRPT2, GCM2, CCND1, RET, CASR, CDKN1B, tumor suppressor, oncogene

1. Introduction

The inappropriate or excessive secretion of parathyroid hormone (PTH) from one or multiple abnormal parathyroid glands typically results in hypercalcemia and the disorder of mineral metabolism called primary hyperparathyroidism (HPT) [1]. Most cases of HPT are sporadic (~95%). Among the small remaining fraction of patients with an inherited basis for HPT, most harbor germline mutation of a known parathyroid tumor susceptibility gene (listed in **Table 1**). In spite of their infrequency, study of the genetics of these uncommon inherited syndromes has yielded substantial insight into the etiology of both sporadic and familial parathyroid tumor development. Since the release of PTH from parathyroid cells involves close regulation by the calcium-sensing receptor (CASR), a cell surface transmembrane receptor of the G protein-coupled receptor family C [2], the germline mutation of the CASR and other genes mediating its signaling can also result in inherited syndromes characterized by hypercalcemia and circulating levels of PTH that are elevated or inappropriately normal. This chapter will summarize current knowledge

Gene	Corresponding protein	Chromosomal location	Associated hyperparathyroid syndrome: main syndromic manifestations	Features of syndromic parathyroid tumors
MEN1	Menin	11q13.1	Multiple endocrine neoplasia type 1 (MEN1): anterior pituitary, parathyroid, enteropancreatic, foregut carcinoid tumors	Multiple, asymmetric tumors typical (>99% benign)
CDC73/ HRPT2	Parafibromin	1q31.2	Hyperparathyroidism-jaw tumor syndrome: fibro-osseous jaw, parathyroid, uterine tumors; renal cysts	Single tumor common (~20% malignant)
CDKN1B	P27(Kip1)	12p13.1	Multiple endocrine neoplasia type 4 (MEN4): anterior pituitary, other involvement varies	Single to multiple glands (benign in reports to date); can be recurrent
GCM2	Glial cells missing transcription factor 2	6p24.2	Familial isolated primary hyperparathyroidism	Single to multiple glands
CASR	Calcium-sensing receptor	3q13.33-q21.1	Familial hypocalciuric hypercalcemia type 1 (FHH1) with heterozygous inactivation; neonatal severe hyperparathyroidism (NSHPT) with homozygous inactivation	FHH1: near-normal size and surgical pathology; altered serum calcium set-point for PTH release NSHPT: marked enlargement of multiple glands by polyclonal (non-neoplastic) mechanism
GNA11	G protein α11 subunit	19p13.3	Familial hypocalciuric hypercalcemia type 2 (FHH2)	ND
AP2S1	Adaptor protein-2 sigma subunit	19q13.32	Familial hypocalciuric hypercalcemia type 3 (FHH3): hypercalcemia more severe than in FHH1	ND
RET	c-Ret	10q11.21	Multiple endocrine neoplasia type 2A: medullary thyroid cancer, pheochromocytoma, parathyroid tumors	Single tumor common (>99% benign)
CCND1/ PRAD1	Cyclin D1	11q13.3	NA (to date, only implicated in sporadic parathyroid tumors)	NA (to date, only implicated in sporadic parathyroid tumors)

Table 1.
Genes implicated in syndromic parathyroid neoplasia and related hypercalcemic states.

of the clinical genetics and molecular pathophysiology of HPT that results from both benign and malignant parathyroid gland neoplasia.

2. The evolution of calcium regulation in vertebrates

In sea water the concentration of elemental calcium is approximately 10 mM. As a result, early eukaryotes living in a marine environment had easy access to calcium. Given this abundant supply of extracellular calcium, numerous intracellular processes evolved in simple eukaryotes that depended on this divalent cation. Such calcium-dependent processes were preserved in metazoans. Thus marine chordates and early vertebrate fish depended on calcium for cellular processes such as membrane permeability, neurotransmitter release, intracellular second messenger signaling, muscular contraction, neuromuscular excitability, and the actions of multiple calcium-dependent enzymes. Calcium's particular coordination chemistry facilitated many proteins' ability to reversibly bind divalent calcium ions, thus enabling signaling through such binding [3].

Calcium is much scarcer on land compared to the marine environment. As lobe-finned fish, marine vertebrates believed to be the ancestors of the early amphibians, began to explore the periphery of the terrestrial environment, evolutionary pressure to develop a system of internal calcium balance mounted. A system of internal calcium homeostasis at the organismal level would ensure the continued preservation and function of numerous cellular and tissue operations that vitally depended on calcium.

Metabolically-active trabecular or cancellous bone in lobe-finned fish and associated hematopoietic bone marrow likely co-evolved [4]. These developments probably both lightened overall skeletal mass and provided a reliable internal source of calcium as a basis for calcium homeostasis. The lightening of skeletal mass was critical since lobe-finned fish and early amphibians had to come to terms with full gravitational force in their terrestrial movements, no longer buoyed by surrounding seawater in accordance with Archimedes' principle [5]. The potential significance of the close physical apposition of hematopoietic bone marrow to spongiform bone, inferred from X-ray synchrotron microtomography of fossilized lobe-finned fish humerus [4], is suggested by the realization that osteoclasts, cells uniquely specialized to mobilize ionized calcium via resorption of bone, develop from hematopoietic stem cell precursors [6]. In contrast, osteoblasts, which lay down osteoid and mineralize bone, derive from mesenchymal stem cells which are abundant in non-hematopoietic bone marrow.

Although analogs of Gcm2, Gata3, CaSR, PTH, and other genes associated with the development and function of human parathyroid glands are expressed in the fish gills, actual parathyroid glands are first seen in amphibians [7–9]. Complete surgical excision of parathyroid gland tissue in amphibians, reptiles, birds, and mammals results in tetany and death.

3. The pathophysiology of primary hyperparathyroidism

PTH secretion from cells of the parathyroid glands is finely regulated in response to changes in the ambient ionized calcium level in order to maintain the circulating calcium concentration within a defined physiologic range. The G protein-coupled CASR is a critical regulator of PTH secretion and is located on the plasma membrane of chief cells in the parathyroid glands [10, 11]. In a classic endocrine negative feedback loop, the active form of cholecalciferol,

1,25-dihydroxyvitamin D, whose synthesis is stimulated by PTH acting on proximal renal tubular cells, inhibits PTH biosynthesis and release from parathyroid cells [12–15]. The simultaneous demonstration of elevated serum calcium with an inappropriately normal or elevated PTH is a typical clinical definition of HPT [16]. The vast majority of parathyroid tumors are adenomas (i.e. benign tumors), with parathyroid cancer accounting for less than 1% of HPT in most series.

Most cases of HPT are sporadic with inherited forms of HPT representing only 2–5% of cases. As illustrated in **Table 1**, research into the molecular pathophysiology of this small subcategory of cases has notwithstanding yielded important understanding with respect to the genes and pathways that promote parathyroid tumorigenesis. Multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2A (MEN2A), the hyperparathyroidism-jaw tumor syndrome (HPT-JT), and familial isolated hyperparathyroidism (FIHP) are the most common inherited disorders associated with HPT [17–21]. Familial hypocalciuric hypercalcemia (FHH) is a related and largely benign autosomal dominant condition characterized by lifelong asymptomatic hypercalcemia. Often mis-diagnosed as HPT, in FHH the PTH-dependent hypercalcemia does not correct with partial or even subtotal parathyroidectomy [22]. The relevance of these inherited disorders to the underlying molecular pathogenetic alterations in parathyroid tumorigenesis will be discussed in more detail below.

4. Oncogenes and proto-oncogenes

Mutant genes that drive cell growth are called oncogenes and represent one potential molecular mechanism for tumor development. Oncogenes are mutationally activated versions of naturally occurring genes, called proto-oncogenes, which under normal conditions positively regulate cell division and/or cell growth [23]. Oncogenes represent gain-of-function mutants or overexpressed forms of proto-oncogenes that can induce cell growth and cell division, often in a tissue-specific fashion, resulting in tumor formation. Proto-oncogenes often encode proteins that are involved in mitogenic signal transduction. In the context of currently recognized familial cancer syndromes, germline mutational activation of proto-oncogenes is rare as an etiology compared to the inactivation of tumor suppressor genes (see below). Constitutive proliferative signaling resulting from the germline activation of most proto-oncogenes would presumably be deleterious to embryonic and fetal development.

5. The role of tumor suppressor genes in tumor development

Alfred Knudson proposed another model for tumor development based on the study of retinoblastoma disease patterns nearly 50 years ago [24]. Sporadic retinoblastoma is usually monocular. Familial retinoblastoma, though rare compared to the sporadic form, is more frequently binocular and has a much earlier age of onset. The "two-hit" hypothesis of tumor development, as proposed by Knudson, hypothesizes that two events (or "hits") in a parental cell confer a selective growth advantage and result in that cell's clonal expansion [25].

Newer clinical and molecular genetic insight that has emerged since his original proposal allow us to update Knudson's concept. In many hereditary tumor syndromes, an inherited germline DNA mutation that affects one copy of a tumor suppressor gene represents the first "hit" or event and is present throughout all cells of the affected offspring. The greater likelihood of any particular cell acquiring a

"second hit", i.e. a somatic mutation in the second allele of the same tumor suppressor gene that was heretofore unaffected, accounts for the earlier age of onset and predisposition for bilateral and multifocal disease in hereditary tumor syndromes. This "second hit" in somatic DNA, that disables the remaining wild-type allele, typically results from a deletion that involves a portion or the entirety of a chromosome. In the familial tumor syndromes MEN1 and HPT-JT, inactivating mutation that involves both alleles of the *MEN1* and the *CDC73/HRPT2* tumor suppressor genes, respectively, can often be found in parathyroid tumor-derived DNA. In such patients, the first "hit", namely a loss-of-function mutation of the relevant tumor suppressor gene, can frequently be demonstrated in the germline DNA.

6. Multiple endocrine neoplasia type 1 (MEN1)

MEN1 is the most common hereditary cause of primary hyperparathyroidism [26]. The syndrome of MEN1 is characterized by the predisposition to develop tumors derived from cells in the anterior pituitary, parathyroid glands, and endocrine cells present in the gut and pancreatic islets (such as gastrinomas, and pancreatic neuroendocrine tumors such as insulinomas) [27]. Tumors in several other endocrine organs and non-endocrine tumors such as lipomas, angiofibromas, and leiomyomas affecting the esophagus, uterus, and/or ureters for example, can also be associated with the syndrome [27]. HPT is the most penetrant hormonal feature of MEN1.

Familial MEN1 is characterized by autosomal dominant transmission. The predisposition to tumor development in one of the tissues characteristically involved in the MEN1 syndrome is caused by germline inactivating mutation in one copy of the *MEN1* gene on chromosome 11q13 [28]. As of 2015, 576 unique germline mutations in *MEN1* were reported from patients and families with MEN1 [29]. The study of DNA derived from pituitary, parathyroid, and entero-pancreatic tumors from MEN1 patients has shown that most syndromic tumors possess an acquired deletion or other inactivating mutation of the second, wild-type *MEN1* allele [18, 30]. Approximately 10% of patients with MEN1 on a clinical basis are germline *MEN1* mutation-negative.

Conventional DNA sequencing of tumor DNA has identified somatic *MEN1* mutation in up to 35% of sporadic parathyroid adenomas [31–35]. In studies testing for loss-of-heterozygosity (LOH) in sporadic parathyroid adenomas, the frequency of LOH at the *MEN1* locus on chromosome 11q13 ranged from 26 to 37%. Using whole exome sequencing (WES) methodology, somatic *MEN1* mutation was found in some 35% of parathyroid benign tumors, comparable to results using conventional Sanger DNA sequencing [36, 37]. As mentioned above, HPT is the most penetrant feature of MEN1 and is usually the initial manifestation. As a result, true MEN1 families may sometimes be initially mis-assigned a clinical diagnosis of familial isolated hyperparathyroidism (FIHP) if only younger affected members are considered at the time that the family is ascertained (see **Figure 1**).

Mutation of the *MEN1* gene is only rarely associated with parathyroid carcinoma. The occurrence of parathyroid carcinoma in the context of familial MEN1 is extremely uncommon. Fewer than 20 patients with HPT due to parathyroid cancer in the context of the MEN1 syndrome have been reported [38]. LOH analysis of parathyroid tumor-extracted DNA has shown that DNA loss at the location of the *MEN1* gene on chromosome 11q, though frequently seen in benign parathyroid tumors, is quite uncommon in parathyroid carcinomas [39]. Recent studies that use next-generation WES of tumor-derived DNA to profile parathyroid cancers did not report any somatic mutations in *MEN1* [40, 41].

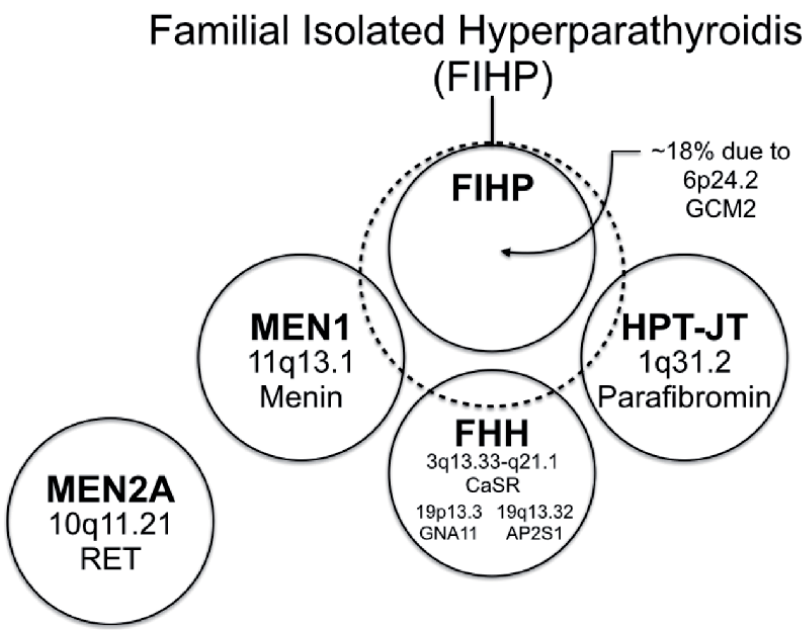

Figure 1.
The relationship among familial forms of hyperparathyroidism that may present as familial isolated hyperparathyroidism (FIHP) as a Venn diagram. The dashed circle represents the set of patients that can present with a provisional diagnosis of FIHP at the time of initial ascertainment. This includes patients with FIHP who have been evaluated for, but lack findings diagnostic of, MEN1, FHH and HPT-JT (nonsyndromic FIHP; in a solid circle). Approximately 18% of nonsyndromic FIHP kindreds harbor germline gain-of-function mutations in GCM2 (see text), whereas the remainder have currently unknown genetic etiologies. Subsets of patients with incomplete expression of MEN1, FHH and HPT-JT (the total set of patients in each syndrome represented by a solid circle) can also present with the FIHP phenotype (and thus overlap with the dashed circle). The distinction between the nonsyndromic FIHP category and the syndromic categories arbitrarily depends on the thoroughness of evaluation and the sensitivity of diagnostic tests used to detect the syndrome that can include germline gene mutational testing. MEN2A is a familial form of hyperparathyroidism that seldom if ever presents as FIHP. Within each circle representing a defined syndrome are included the genetic locus (or loci in the case of FHH; see text) of the syndromic trait and the associated gene product. The causative gene for HPT-JT encoding parafibromin is CDC73, formerly called HRPT2. The relationship among the patient sets illustrated as circles in this diagram is intended to be qualitative and neither the area of each circle nor the area of overlap between circles has any quantitative significance.

7. The hyperparathyroidism-jaw tumor syndrome (HPT-JT)

HPT-JT is a familial syndrome with variable and incomplete penetrance transmitted in an autosomal dominant fashion. The key clinical features of HPT-JT include HPT, jaw tumors (fibro-osseous tumors involving the maxilla and/or mandible, formally classified as cemento-ossifying fibromas [42], and distinct from so called "brown" tumors sometimes associated with HPT), renal cysts or tumors and uterine tumors in women [43–45]. HPT is the most penetrant feature of HPT-JT and is usually the presenting manifestation. In contrast to MEN1, parathyroid cancer is frequent in HPT-JT, affecting some 20% or more of those with HPT [43–46].

In the majority of HPT-JT kindreds, a germline loss-of-function mutation of the *CDC73* gene (formerly called *HRPT2*) can be identified [19, 47]. The majority of such *CDC73* mutations are predicted to inactivate gene function via frameshift or nonsense mutation, and only a minority of the mutations are missense [48]. Patients and kindreds with partial or complete deletion of the *CDC73* gene in the germline have also been described [49–52]. The *CDC73* gene encodes a 531-residue protein named parafibromin [47]. Because germline mutation predicted to cause loss-of-function of the *CDC73* gene predisposes to the neoplastic expressions

of HPT-JT, parafibromin is considered to be a tumor suppressor protein. Mixed epithelial tumor of the kidney (MEST), a rare type of renal tumor (formerly classified as cystic hamartoma of the renal pelvis, leiomyomatous renal hamartoma, or adult type mesoblastic nephroma), has been associated with HPT-JT and appears to correlate with a specific *CDC73* genotype, namely the Met1ILe missense mutation replacing the initiator methionine of parafibromin with isoleucine [47, 53, 54]. Somatic mutation of the *CDC73* tumor suppressor gene is uncommon in sporadic parathyroid adenomas [55]. In contrast to the results of analyses in benign parathyroid tumors, mutations of *CDC73* are quite frequently seen in apparently sporadic cases of parathyroid cancer [56–58]. Interestingly, recurrent somatic mutations in *CDC73* have been documented by exome sequence analysis of tumor DNA from parathyroid cancers [40, 41]. Selective amplification of the mutant copy of *CDC73* has been demonstrated in a subset of parathyroid carcinomas [40]. Approximately 25% of cases of seemingly sporadic parathyroid carcinoma may possess germline loss-of-function alterations in *CDC73*, suggesting that such patients may in fact have previously unrecognized, or *formes frustes* of, HPT-JT [19, 57, 58]. A minority of patients and families classified as FIHP can be shown to carry *CDC73* mutation in the germline, suggesting that this inherited disorder may in some cases be phenocopied by incompletely penetrant HPT-JT (see below and **Figure 1**). Approximately 20% of genetically confirmed or obligate *CDC73* mutation-positive family members lack HPT, fibro-osseous jaw tumors, or other manifestations of HPT-JT when their kindred is initially ascertained. Because the penetrance of the manifestations of HPT-JT increases with age among *CDC73* mutation carriers, lifelong surveillance of initially asymptomatic carriers is recommended [59].

8. Multiple endocrine neoplasia type 4 (MEN4)

MEN4 is a syndrome originally described by Pellegata and coworkers in a multigenerational family with features resembling MEN1, including a proband with a growth hormone-secreting pituitary adenoma and HPT, but lacking germline *MEN1* mutation [60, 61]. A germline heterozygous truncation mutation in *CDKN1B* was identified in the proband and several members of this kindred [60]. *CDKN1B* encodes the cyclin dependent-kinase inhibitor p27 (Kip1). Attention to the *CDKN1B* locus was a consequence of a previous genetic analysis of rats with the MenX phenotype, a recessively inherited condition caused by a frameshift mutation in *Cdkn1b* [60, 62]. The MenX phenotype in rats was manifest by the development of bilateral pheochromocytomas, paragangliomas, parathyroid adenomas and thyroid C cell hyperplasia [60, 62]. In the study by Pellegata et al., the proband was the only member of the MEN4/MENX kindred described who manifested HPT [60].

Following the original report by Pellegata et al. [60], several groups have investigated a possible role for *CDKN1B* mutation in parathyroid tumorigenesis. None of the earlier reports of *MEN1* mutation-negative families harboring germline mutation in *CDKN1B*, and expressing MEN1-like tumors and thus classified as MEN4, had included families with more than one member with HPT proven to track with the *CDKN1B* mutation [60, 63–71], apart from the demonstration of HPT linked to *CDKN1B* mutation in monozygotic twins [64]. That was true until a more recent report by Frederiksen et al. describing a large Danish family in which HPT occurred in 13 members, spanning two generations, who carried a germline frameshift *CDKN1B* mutation [72].

Recent evidence supports the characterization of *CDKN1B* as a susceptibility gene for the development of primary parathyroid tumors [69, 72, 73]. This evidence validates the inclusion of germline *CDKN1B* mutation in the differential diagnosis

of familial HPT, particularly in the evaluation of germline *MEN1* mutation-negative families who yet have MEN1-like features. The strongest justification for this follows from consideration of the Danish kindred in which 13 unique family members manifest HPT linked to germline inactivating mutation of *CDKN1B*, described by Frederiksen and co-workers [72].

9. Familial isolated hyperparathyroidism (FIHP)

By definition, FIHP is a non-syndromic category of familial HPT describing families that contain two or more members with HPT but which lack the specific features of MEN1, MEN2A, HPT-JT or FHH (**Figure 1**) [74]. FIHP is genetically heterogeneous and is a diagnosis of exclusion. While at the time of initial ascertainment germline mutation of *MEN1*, *CDC73*, or *CASR* may account for a fraction of kindreds with the FIHP phenotype [20, 34, 75–77], the majority of FIHP families lack mutations in these established HPT-susceptibility genes (**Figure 1**) [20, 75, 78].

Missense variants in GCM2, a transcription factor homologous to the Drosophila "glial cells missing" (gcm) gene and required for parathyroid gland development, were recently described in the germline DNA of eight unrelated families with FIHP [21]. Previous studies showed that germline dominant-negative and loss-of-function mutations in GCM2 were associated with autosomal dominant and autosomal recessive familial isolated hypoparathyroidism, respectively [79, 80]. The two rare germline *GCM2* genetic variants associated with FIHP act as gain-of-function mutations [21]. These missense mutations map to the C-terminal conserved inhibitory domain (CCID) of GCM2 and increase its transcriptional activity when measured *in vitro*, suggesting that *GCM2* in the context of FIHP is a parathyroid proto-oncogene. It has been estimated that approximately 18% of FIHP families harbor germline activating GCM2 mutations [21], leaving ~80% of FIHP families without a currently-identified genetic etiology [74]. Other clinical investigators have identified rare germline *GCM2* variants in a subset of FIHP kindreds [81]. Activating *GCM2* variants mapping to the CCID region have been found among patients with sporadic parathyroid tumors in low frequency and appear to be of low penetrance [82].

10. Familial hypocalciuric hypercalcemia (FHH)

FHH is a condition of PTH-dependent hypercalcemia, often resembling HPT, that is clinically benign and genetically heterogeneous (**Table 1**) [22]. Following partial or subtotal parathyroidectomy, affected patients from FHH kindreds almost always remain hypercalcemic. FHH is transmitted in an autosomal dominant fashion and usually causes mild hypercalcemia with relative hypocalciuria. The hypercalcemia seen in FHH is highly penetrant across all ages, including in infants [22, 83]. The majority of cases of FHH result from heterozygous germline inactivating mutation of the *CASR* gene on the long arm of chromosome 3 that encodes the calcium-sensing receptor [10, 84], and is classified as type 1 FHH (FHH1). Neonatal severe hyperparathyroidism (NSHPT), a rare autosomal recessive disorder typically presenting with severe hypercalcemia occurring in the first 6 months of life, most often results from the compound heterozygous or homozygous inheritance of two loss-of-function mutant *CASR* alleles [85]. Rather than the cellular monoclonality that would be expected in true parathyroid tumors, molecular genetic analysis of the hyperfunctioning parathyroid glands removed from a patient with NSHPT

demonstrated generalized polyclonal hyperplasia, underscoring the non-neoplastic nature of the abnormal parathyroid glands associated with *CASR* inactivating mutation [86].

Loss of surface expression of the CASR protein has been documented in parathyroid adenomas and may contribute to the altered calcium set point and impaired calcium-mediated negative feedback on the release of PTH typical of such adenomas. Decreased *CASR* mRNA expression, but not LOH at the *CASR* locus, has been documented in parathyroid adenomas [87]. In sporadic parathyroid tumors studied to date, somatic inactivation of the *CASR* gene has not been reported [88, 89].

Type 2 FHH (FHH2) resulting from germline loss-of-function mutation of *GNA11*, encoding the G protein α11 subunit [90, 91], and type 3 FHH (FHH3) resulting from germline inactivating mutation in *AP2S1*, the gene that encodes an adaptor protein involved in endocytosis mediated by clathrin [92–95], have also been described. In studies of sporadic parathyroid tumors, somatic inactivating mutations of *GNA11* and *AP2S1* have so far not been reported.

11. Multiple endocrine neoplasia type 2A (MEN2A)

MEN2A is a familial cancer syndrome characterized by a predisposition to the development of medullary thyroid cancer (MTC), pheochromocytoma (typically benign and often bilateral), and primary HPT. In the context of MEN2A, HPT is usually mild and resembles sporadic HPT. HPT in MEN2A is almost always results from benign parathyroid disease. MEN2A is an autosomal dominant disorder that results from germline gain-of-function mutation in the *RET* proto-oncogene at chromosomal location 10q11. *RET* encodes a receptor tyrosine kinase that binds the ligand glial derived neurotrophic factor, together with a glycosylphosphatidylinositol-anchored protein co-receptor Gfra1 [96].

Germline oncogenic mutations of *RET* are associated with three distinct familial endocrine neoplasia syndromes, all associated with MTC: MEN2A, multiple endocrine neoplasia type 2B (MEN2B), and familial medullary thyroid cancer (FMTC). The disease spectrum of typical MEN2B or FMTC does not include parathyroid tumors and HPT. Genotype–phenotype correlations based on particular *RET* mutations are apparent and account for the distinct patterns of disease. Some 95% of MEN2A cases are due to the presence in the germline of nonsynonymous variants affecting the RET receptor's extracellular cysteine-rich domain, namely missense mutations of *RET* codons 609, 611, 618, 620, or 634 [97]. In fact, germline missense alteration of RET residue cysteine-634 accounts for approximately 85% of cases of MEN2A [98].

12. Parathyroid tumorigenesis involving the CCND1 oncogene

The discovery of the *CCND1* (or *PRAD1*, for parathyroid adenomatosis 1) oncogene resulted from the analysis of several large, non-familial, parathyroid adenomas that harbored DNA re-arrangements that involved the PTH gene locus [99–101]. A breakpoint resulting from the pericentromeric inversion of chromosome 11 DNA was identified just upstream of the *CCND1/PRAD1* oncogene [101]. The inversion positioned the PTH gene regulatory region, that is normally located on the short arm of chromosome 11, just upstream of the *CCND1/PRAD1* proto-oncogene located on 11q [99–101]. The product encoded by the proto-oncogene

was subsequently recognized by DNA sequence analysis to be a member of the cyclin protein family [101]. The gene was therefore re-named cyclin D1 (*CCND1*). Overexpression of *CCND1* in the parathyroid cells of transgenic mice induces cell proliferation and gives rise to the metabolic abnormalities that characterize HPT in humans [102].

While activating CCND1 missense mutations have not been observed in sporadic parathyroid tumors [103], overexpression of *CCND1* has been demonstrated in 20–40% of sporadic benign parathyroid tumors and in an even larger percentage of parathyroid carcinomas [104–107]. In parathyroid carcinoma, no somatic chromosomal rearrangements on chromosome 11 involving *CCND1* have been reported. Neither germline activating missense mutations of *CCND1* nor chromosomal translocations or rearrangements involving the *CCND1* locus have been reported in any familial form of HPT.

13. Other genes involved in parathyroid tumorigenesis

Recurrent mutations in a subset of genes likely relevant to parathyroid tumorigenesis have been identified by WES analysis of DNA derived from sporadic parathyroid tumors. Eight out of 193 sporadic parathyroid tumors analyzed by WES demonstrated the Y641N missense mutation in the *EZH2* gene on chromosome 7 that encodes the enhancer of zeste 2 polycomb repressive complex 2 subunit [36]. Analysis by WES of 22 parathyroid tumors derived from a Chinese patient population identified a distinct somatic missense mutation, Y646N, in *EZH2* [108]. Acquired mutations of Y641 and Y646 in *EZH2* were described previously in lymphoid malignancy [109, 110]. Molecular genetic profiling of 80 sporadic parathyroid neoplasms by separate investigators failed to uncover any pathogenic *EZH2* mutations however, suggesting acquired *EZH2* mutation may be uncommon in parathyroid tumors [111]. In the context of lymphoma, *EZH2* is thought to function as a proto-oncogene [109]. To date, no transgenic mouse models restricting *EZH2* mutation or overexpression to parathyroid cells have been reported.

Soong and Arnold used WES analysis of DNA extracted from 19 parathyroid adenomas and matching germline DNA to identify somatic mutations in *ZFX*, a putative parathyroid proto-oncogene and member of the Krüppel associated box domain-containing family of zinc finger protein transcription factors [112]. Their observations in the discovery cohort were confirmed by direct sequencing of tumor DNA from an additional validation set comprised of 111 parathyroid adenomas [112]. The mutant *ZFX* alleles detected in parathyroid tumors likely act as oncogenes [113]. Such somatically acquired ZFX mutations in parathyroid tumors may be uncommon, however, since an independent mutational analysis of 23 sporadic parathyroid carcinomas and 57 adenomas failed to identify any pathogenic *ZFX* variants [111]. The development of a transgenic mouse model and/or better characterization of the functional properties of the mutant ZFX protein may clarify the potential significance of *ZFX* as a parathyroid proto-oncogene.

WES analysis of 22 blood-sporadic parathyroid adenoma tumor pairs from a Chinese patient cohort identified recurrent mutations of *ASXL3* [108]. ASXL3 belongs to a family of vertebrate Additional sex combs (Asx)-like proteins that may function as regulators of transcription. It remains unclear if the somatic missense *ASXL3* mutations identified in the parathyroid adenomas, mutations that affected highly conserved residues, would result in gain- or loss of ASXL3 function [108]. Further studies will be required to confirm this initial observation and to clarify the mechanism by which *ASXL3* mutation might drive parathyroid tumor development.

14. Conclusions

While inherited forms of HPT represent only a small fraction of cases (<5%), study of the molecular pathophysiology of these uncommon familial syndromes has yielded substantial insight into the genetic etiology of both sporadic and familial parathyroid disease and resulted in the identification of genes such as *MEN1, CDC73, CASR, GNA11, AP2S1, CDKN1B, CCND1*, and *GCM2*. It is highly likely that the mutational gain- or loss-of-function of other, yet unrecognized, genes is able to drive parathyroid neoplasia. For example, the risk in the majority of FIHP kindreds predisposing to the development of parathyroid tumors seems to result from the germline mutation of genes not presently recognized as having a role in parathyroid disease. This follows from the observation that nearly 70% of families initially considered as FIHP in multiple studies that examined for germline *MEN1*, *CASR* and *CDC73/HRPT2* gene mutation, had no recognized syndromic etiology (**Figure 1**) [20, 75–77]. From among those FIHP kindreds who are *MEN1*, *CASR* and *CDC73* mutation-negative, only about 20% are estimated to harbor germline activating mutations in the *GCM2* proto-oncogene [21], which leaves nearly 80% of FIHP kindreds with a currently-undefined genetic basis for their disease (**Figure 1**).

The existence of currently unidentified parathyroid tumor suppressors and oncogenes is also suggested by analysis of parathyroid tumors using techniques such as comparative genomic hybridization (CGH) to identify specific chromosomal regions harboring loss or gain of DNA. Several investigators have documented recurrent loss of DNA at the 1p, 6q, 9p, and 13q chromosomal loci in parathyroid tumors, indicating the potential presence there of novel parathyroid tumor suppressor genes [114–117]. The potential presence of novel oncogenes at chromosomal loci 9q, 16p, 19p, and Xq is suggested by results demonstrating specific chromosomal gain at these loci in benign or malignant parathyroid tumors [114, 116–118].

Next-generation sequencing analysis including WES of parathyroid neoplasms is an auspicious approach for the identification of novel acquired and germline gene variations that predispose to the development of HPT and parathyroid neoplasia. The apparent validation of this line of investigation by the identification of *EXH2* [36], *ZFX* [112], and potentially *ASXL3* [108], as candidate driver genes for parathyroid neoplasia was previously discussed. WES analysis of parathyroid cancer-derived DNA has similarly underscored the possible significance of recurrent somatic and germline inactivating mutations of *PRUNE2* in the etiology of parathyroid malignancy [40]. The comprehensive quality and great sensitivity of WES and related next-generation sequencing methodologies should further advance our insight into the genetic basis and endocrine pathophysiology of inherited and sporadic parathyroid neoplasia in the decades ahead.

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Conflict of interest

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Author details

William F. Simonds
Metabolic Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA

*Address all correspondence to: bills@niddk.nih.gov

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Section 5

Electrolyte Disturbances

Chapter 9

Electrolytes in the ICU

Syed Zaidi, Rahul Bollam and Kainat Saleem

Abstract

Electrolyte disorders is an imbalance of certain ionized salts (sodium, potassium, calcium, bicarbonate, chloride) in the blood. Healthcare providers should be familiar with the principles of electrolyte physiology and pathophysiology. Disturbances in sodium homeostasis are primarily caused by volume abnormalities leading to primarily neurologic symptoms. Dyskalemias frequently present with cardiac manifestations therefore should be treated promptly before evaluating its cause. Ion deficiencies such as hypocalcemia, hypomagnesemia and hypophosphatemia should be corrected as they are associated with increased adverse events in critically ill patients.

Keywords: Electrolytes, Sodium, Potassium, critically ill, ICU

1. Introduction

Electrolytes are elements that naturally occur in the human body and help balance pH, facilitate passage of fluid through osmosis and regulate the function of neuromuscular, endocrine and excretory systems. Disturbances in these electrolytes are common clinical problems encountered in the intensive care unit with serious complications when they are depleted. Recent studies report that electrolyte imbalances are associated with increased morbidity and mortality. Possible mechanisms include damage to the kidney, activation of hormonal systems (such as RAAS) or the myriad of medication given in a critically ill patient. This chapter will focus on various electrolyte abnormalities seen in the critical care setting then touch on important ICU scenarios which could affect electrolytes.

2. Disorders of sodium homeostasis

- Serum sodium reflects the plasma tonicity and is inversely related to total body water. Changes in sodium are generally due to changes in total body water, not serum sodium, which regulates plasma tonicity and effective arterial volume. The body normally prevents plasma sodium to stray outside normal range (135 to 145 mEq/L or mmol/L) by controlling water intake and excretion.

- Sodium crosses systemic capillary membranes through clefts between endothelial cells therefore sodium concentration is identical in plasma and interstitial fluid [1]. Brain capillaries have tight endothelial junctions lined by astrocytic foot processes creating the blood–brain barrier which sodium cannot cross [2]. Consequently, an abnormal plasma sodium causes water movement across the blood brain barrier leading to either brain swelling or shrinkage.

- Osmoreceptors are hypothalamic neurons that are responsible for adjusting thirst and vasopressin secretion based on plasma sodium. Vasopressin binds to V2 receptors on the principal cells lining the renal collecting duct [3]. In the presence of vasopressin, water is allowed to flow out of the collecting tubule in the nephron attracted by a high solute concentration of the surrounding medullary interstitium. When plasma sodium is high, vasopressin levels are also increased.

2.1 Hyponatremia

2.1.1 Introduction

- Hyponatremia is defined as serum sodium below 135 mEq/L (135 mmol/L). It is the most common electrolyte disorder seen in clinical practice and the consequences can range from minor symptoms to life threatening complications including seizures and cardiorespiratory distress. 30% of critically ill patients have serum sodium<134 mEq/L [4]. Patients with hyponatremia are seven times more likely to die than those without hyponatremia during hospitalization [5].

- Hyponatremia can be classified based on temporality (acute <48 hrs; chronic >48 hrs) or based on serum sodium (mild 130-135 mEq/L (mmol/L); moderate 125-130 mEq/L (mmol/L); severe <125 mEq/L (mmol/L)) [6].

2.1.2 Presentation

- Patients with mild to moderate hyponatremia can present with nausea, confusion, headaches whereas moderate to severe hyponatremia can presenting with vomiting, cardiorespiratory distress, seizures and reduced consciousness [6].

- Patients are likely to present with severe symptoms if they have acute onset hyponatremia while chronic hyponatremia has a lower risk of neurological dysfunction as the brains counter-regulatory mechanisms [7].

2.1.3 Diagnostic approach

- The diagnostic approach of hyponatremia should follow a logical progression, answering several key questions. See **Figure 1**.

2.1.4 Treatment

- Treatment of hyponatremia is based on underlying pathogenesis.

- In patients who present symptomatically with hyponatremia, they should be considered as an emergency and given hypertonic therapy. 3% normal saline can be used, 150 ml over 20 min. Lab work should be obtained after hypertonic therapy is administered and the goal is to increase sodium by 3-5 mM which should improve patient's symptoms. If patients symptoms persist and sodium has increased by <4 mM then another round of hypertonic therapy can be given. If sodium increased by >6 mM and symptoms have not resolved, then further workup should be performed to check for alternative pathologies. Hypertonic bicarbonate has the same tonicity as 6% NaCl and is usually

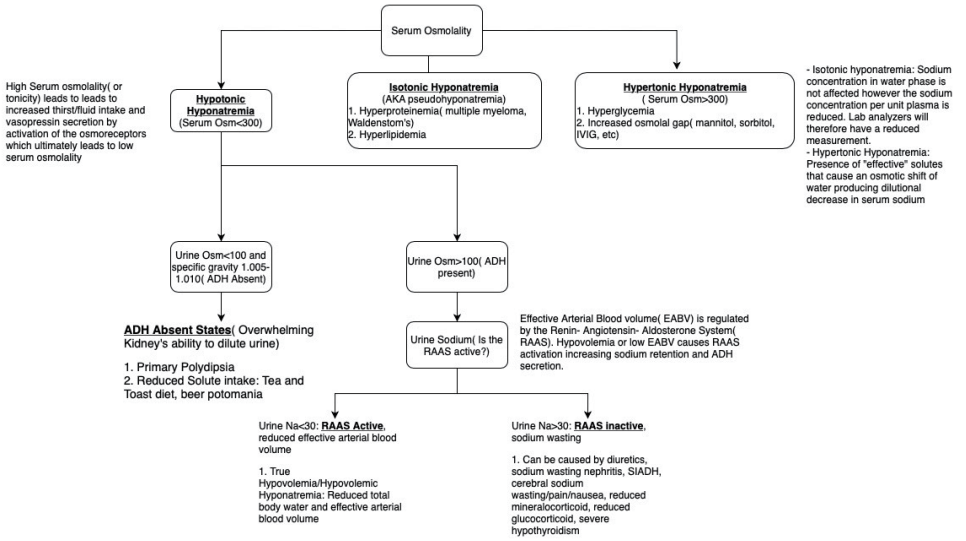


Figure 1.
Diagnostic approach to hyponatremia, starting with osmolality.

the fastest medication to obtain in an emergency. Typical dose of two ampules of hypertonic bicarbonate is equivalent to ~200 ml of 3% saline [8]. See **Figure 2**.

- Depending on the pathology, hyponatremia will be treated differently [9].

- In patients with ADH absent states, hyponatremia is caused because patient is drinking more fluid than the kidney can handle. In primary polydipsia, fluid restriction would be ideal [9].

- Normally the kidneys require solute to create urine therefore in patients with poor nutritional status, a normal amount of water/alcohol will cause hyponatremia. In patients with reduced dietary solute intake (such as chronic alcoholics), instituting a proper diet will correct hyponatremia. These patients can be given isotonic fluid is clinical evidence of hyponatremia [9].

- ADH absent states are high risk for over-correction therefore should be monitored closely.

$$Maximum\ Urine\ Output = \frac{Dietary\ Solute\ Intake}{Urine\ Osmolality} \tag{1}$$

Normal diet contains 600-900 mosmol of solute/day and the minimum urine osmolality is 60 mosmol/kg therefore the maximum urine output (see Eq. 1) in a normal patient would be:

$$Maximum\ Urine\ Output = \frac{900}{60} = 15\ litres\ per\ day$$

Therefore, in patients with primary polydipsia, they will overcome the maximum urine output while patients with reduced solute intake will have reduced maximum urine output.

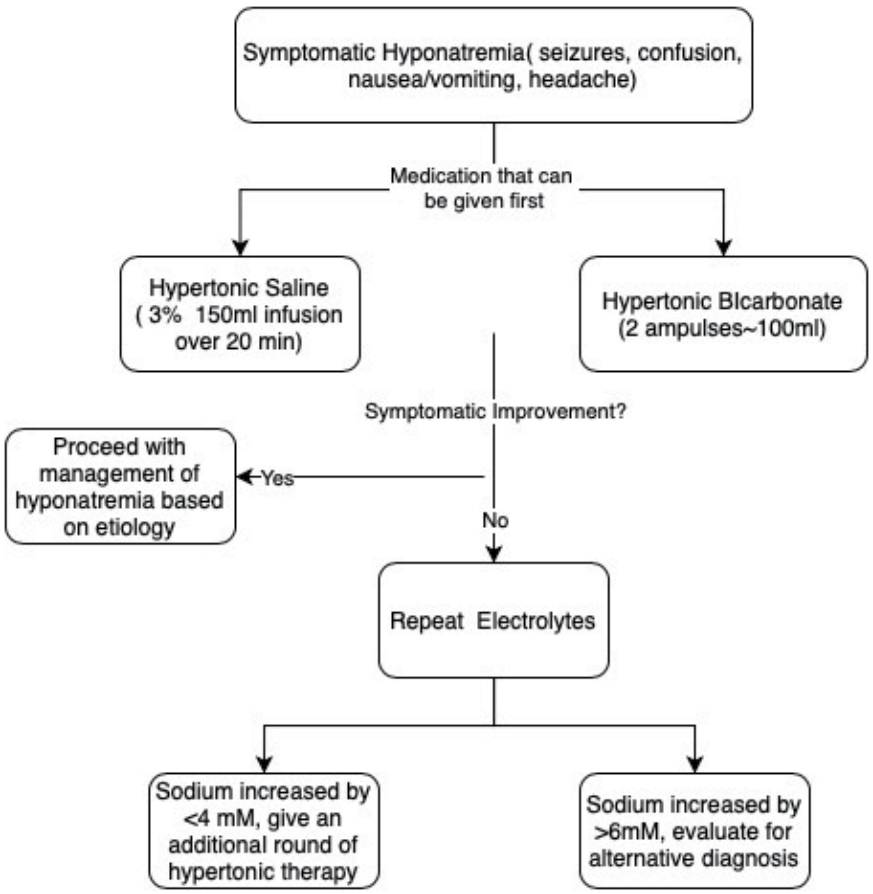


Figure 2.
Treatment algorithm for hyponatremia.

- In patients who have reduced EABV, treating the primary pathology will improve the serum sodium. History and physical examination is important when it comes to this patient population to effectively start correct treatment plan.

- In SIADH, the underlying cause should be treated concomitantly with initial treatment to raise serum sodium. Fluid restriction is the mainstay of therapy. In patients who have chronic SIADH, sodium or urea tablets can be used. Since patients with SIADH have a fixed urine osmolality, solute loads (such as urea and sodium tablets) are used to increase maximum urine output by increasing dietary solute [8, 9].

- In a patient who presents to the hospital with hyponatremia, it will be assumed to be chronic in nature. The target goal for these patients is 6-8 mEq/L (mmol/L) in a 24-hour period. Excessively rapid correction leads to osmotic demyelination syndrome.

- Common causes of hyponatremia overcorrection are:

    1. Treating the underlying cause

    2. Potassium Supplementation

3. Vaptans

- Risk factors for over-correction are cirrhosis, alcoholism, malnutrition and hypokalemia.

- Administration of potassium will also increase plasma sodium because it enters the cells, increasing intracellular osmolality and causing water to move from the extracellular space into the intracellular space, thus raising plasma sodium concentration

2.1.5 Overcorrection of sodium

- In a patient whose sodium was corrected outside 24 hour parameters can be started on rescue strategy. Desmopressin can be given (dose: 2mcg IV/subQ every 6 hours) to reduce free water output. Concomitantly, free water should be given (D5W at 6 ml/kg infused over 2 hours with labs after every infusion to determine rate of lowering) with the goal to bring sodium back within suitable levels for the next 24 hours. Once the sodium goal is achieved, the D5W can be stopped but desmopressin can be continued to prevent overly rapid correction [8, 9].

- Osmotic Demyelination syndrome: This syndrome usually has a delayed presentation 2-6 days after over-correction. Symptoms are dysarthria, dysphagia, paresthesia, quadriparesis and seizures. These symptoms are irreversible. MRI brain will show demyelinating lesions however may not appear for atleast 4 weeks after disease onset. Earlier detection may be possible with newer techniques such as DWI [8, 9].

2.2 Hypernatremia

2.2.1 Introduction

- Hypernatremia is defined as >145 mEq/L (mmol/L). This is often seen in hospitalized patients and is associated with increased mortality in patients [10–12]. Hypernatremia represents a deficit of water in relation to the body's sodium stores which can result from a net water loss or a hypertonic sodium gain.

2.2.2 Clinical presentation

- Most patients with hypernatremia are either very young or old [13]. Signs usually reflect central nervous system dysfunction. Elderly patients generally have few symptoms unless sodium exceeds 160 mEq/L [13, 14].

- Brain shrinkage induced by hypernatremia can cause vascular rupture, with cerebral bleeding, subarachnoid and permanent neurological damage. This is counteracted by a solute movement to the brain which normalizes the brain volume but does not correct hyperosmolality in the brain [15, 16].

- In patient with prolonged hyperosmolality, aggressive treatment with hypotonic fluids may cause cerebral edema [17, 18].

2.2.3 Diagnostic approach

- Net water loss accounts for a majority of cases of hypernatremia [19]. Since sustained hypernatremia can occur only when thirst or access to water is impaired, the groups at highest risk are patients with altered mental status, intubated patients and elderly individuals [20].

- Hypertonic sodium gain usually results from clinical interventions or accidental sodium loading. See **Figure 3** for diagnostic approach.

2.2.4 Treatment

- The treatment of hypernatremia requires addressing the underlying cause and correcting the prevailing hypertonicity.

- In patients who developed hypernatremia is several hours, rapid correction improves prognosis without increasing the risk of cerebral edema. The serum sodium can be reduced by 1 mEq/L (mmol/L) [16].

- A slower rate of correction is required in hypernatremia that lasted longer (≥2 days) or for unknown duration [21]. ln these patients, maximal rate of 0.5 mEq/L/hr. prevents cerebral edema and seizures [22, 23]. The goal of treatment is to reduce the serum sodium to 145 mEq/L.

- The preferred route of administering fluid is enterally however if not feasible then fluids should be given intravenously. Hypotonic fluids such as free water, 5% dextrose, ¼ isotonic saline and ½ normal saline can be used. The more hypotonic the infusate, the slower the infusion rate.

- Once the infusate is chosen, the free water deficit can be calculated (see Eq. 2) [24]:

$$Free\ Water\ Deficit = Total\ Body\ Water\% \times Weight(in\ kgs) \times \frac{Curent\ Sodium}{Ideal\ Sodium - 1} \quad (2)$$

* TBW is:
Adult Male: 60%; 50% in elderly.

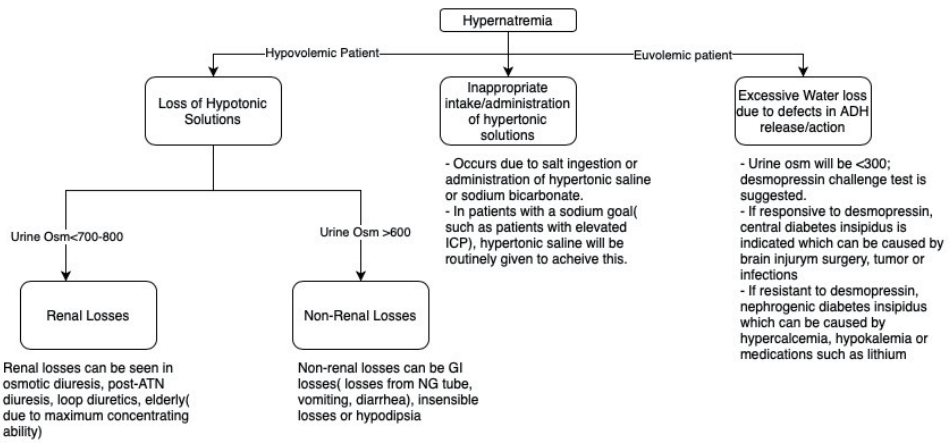


Figure 3.
Diagnostic approach for hypernatremia.

Adult Female: 50%; 45% in elderly.

- Through hospitalization, patients will have ongoing water losses which includes insensible losses (stool, sweat, respirations) and urine free water that should be accounted for. Insensible losses cannot be measured therefore can be approximated as 30-50 ml/hr [25, 26]. Urine free water can be calculated (see Eq. 3):

$$\textit{Urine Free Water clearance}(ml/hr) = \textit{Urine flow rate} - \frac{\textit{Urine flow rate} \times (\textit{Urine sodium} + \textit{Urine Potassium})}{\textit{Serum Sodium}} \quad (3)$$

- For acute or chronic hypernatremia, serum sodium should be measured every 4-6 hours and the estimated fluid replacement rate should be adjusted accordingly.

3. Potassium homeostasis

- Patients is an important electrolyte that has been proven essential for normal functioning of the cardiovascular system, skeletal muscle, internal organs and nervous system.

- The intracellular proportion of K+ represents 98% of the total body K+. The intracellular potassium concentration is approximately 140 mEq/L (mmol/L) compared to the normal serum potassium of 3.5-5.5 mEq/L (mmol/L). This ratio of potassium concentrations in the cells and extracellular fluid is a major determinant of the resting membrane potential across cell membranes [27].

- An abnormal potassium level predisposes patients to serious complications such as cardiac arrythmias, muscle weakness which could provoke sudden cardiac arrest or respiratory failure.

3.1 Hyperkalemia

3.1.1 Introduction

- Hyperkalemia is defined as serum potassium ≥5.5 mEq/L (mmol/L) which is commonly seen in patients with chronic kidney disease, diabetes or cardiovascular disease. High potassium intake is rarely sufficient to result in hyperkalemia [28].

- Based on the European Resuscitation Council Guidelines classification of hyperkalemia based on serum potassium levels [28]:

    1. Mild Hyperkalemia: - Serum Potassium 5.5-5.9 mEq/L (mmol/L)

    2. Moderate Hyperkalemia: - Serum Potassium 6.0-6.4 mEq/L (mmol/L)

    3. Severe Hyperkalemia: - Serum Potassium ≥6.5 mEq/L (mmol/L)

- Hyperkalemia is associated with increased mortality in patients with chronic kidney disease and ESRD on dialysis. See **Figure 4** for causes of hyperkalemia.

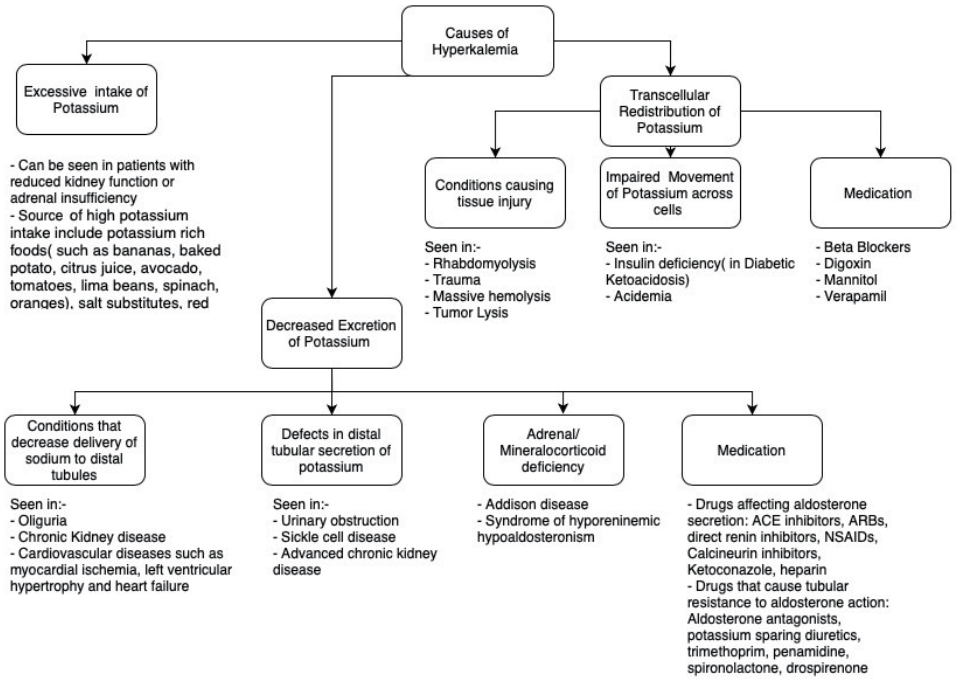


Figure 4.
Major etiologies of hyperkalemia.

3.1.2 Clinical presentation

- Hyperkalemia can manifest with neuromuscular weakness, bradycardia and ventricular tachycardia/fibrillation. In practice however, most patients are asymptomatic [28].

- Hyperkalemia is usually caused by increased potassium intake, decreased renal excretion and transcellular shift in potassium. The various pathologies that could lead to hyperkalemia can be divided based on underlying mechanism of cause:

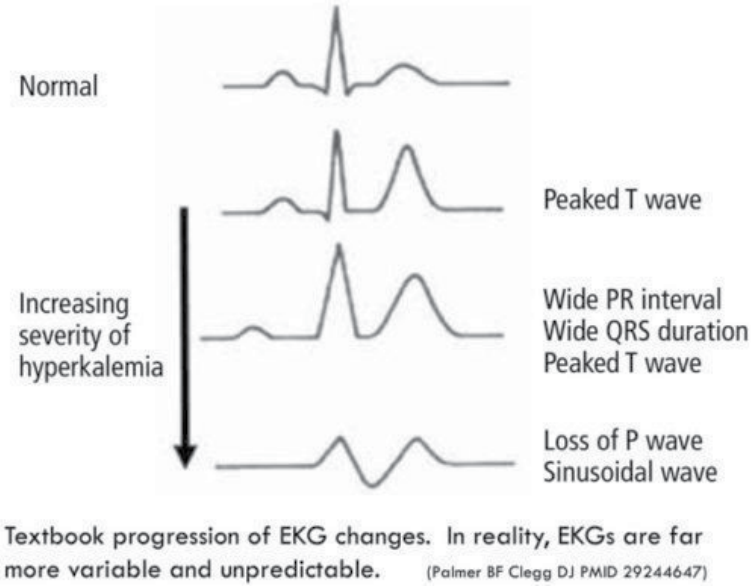
- Pseudo-hyperkalemia refers to artificially elevated potassium which is seen in hemolysis, severe polycythemia and prolonged tourniquet application.

3.1.3 EKG findings

- EKG changes in hyperkalemia are seen with rising serum potassium levels (see EKG 1). Characteristically, there will be:

    1. Peaked T waves, best seen in the precordial leads
    2. Flattened P wave with prolonged PR interval
    3. Absent P wave
    4. Wide QRS and sine wave pattern

- There is a poor correlation between serum potassium levels and cardiac manifestations reported [28, 29].



EKG 1. EKG pattern showing changes in hyperkalemia.

3.1.4 Treatment

- Whenever hyperkalemia is seen on labs, and EKG should be done. If EKG changes are present or patient is symptomatic consistent with hyperkalemia, then this will confirm the diagnosis.

- In practice, most patients with hyperkalemia are asymptomatic (even with severe hyperkalemia).

- Potassium levels >6.5-7 (mmol/L) are more worrisome. Chronic hyperkalemia is better tolerated compared to acute which is more dangerous. Chronic hyperkalemia is seen in dialysis patients who are frequently hyperkalemic

- The acute management of hyperkalemia is the prevention or reversal of cardiac dysrhythmias. The primary goal of chronic treatment of hyperkalemia is to maintain serum potassium levels after acute treatment leads to reduction in serum potassium.

- Acute management of hyperkalemia [28, 30, 31]: See **Figure 5**.

- IV calcium gluconate is preferred over calcium chloride because calcium chloride causes skin irritation and extravasation which can lead to skin necrosis or thrombophlebitis. Peripherally, 3 g IV calcium gluconate can be given over 10 min. For central access, 1 g over 10 min or slow IV push can be done. Calcium lasts for 30-60 mins so it may need to be repeated.

- Regular insulin 10-20 units IV can be given with dextrose 25 g (when blood glucose <250 mg/dl). In patients with renal insufficiency, short acting insulin can be used. Insulin lasts for a few hours therefore may need to be re-dosed.

- 10-20 mg albuterol can be given in normal saline over 10 min with nebulizer.

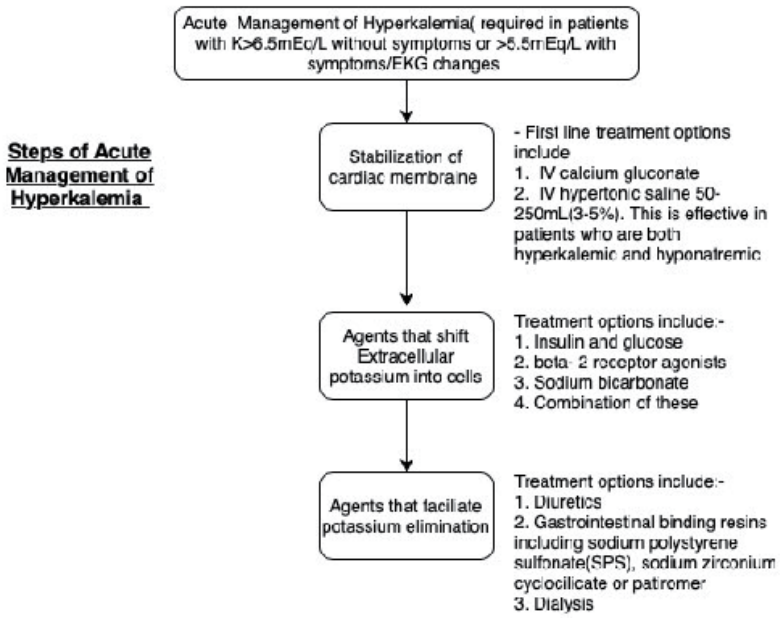


Figure 5.
Steps of acute management of hyperkalemia.

- Ultimately, patients will require elimination of excess potassium from the body (**Figure 6**).

- Diuresis with furosemide is suggested in hypervolemic/euvolemic patients able to produce urine. Furosemide increases urinary excretion of potassium which can be used in both acute and chronic management. Dialysis should be considered in patients who fail medical management, severe AKI/ESRD or persistent EKG changes.

- Chronic Management of hyperkalemia includes maintaining serum potassium after acute treatment [32].

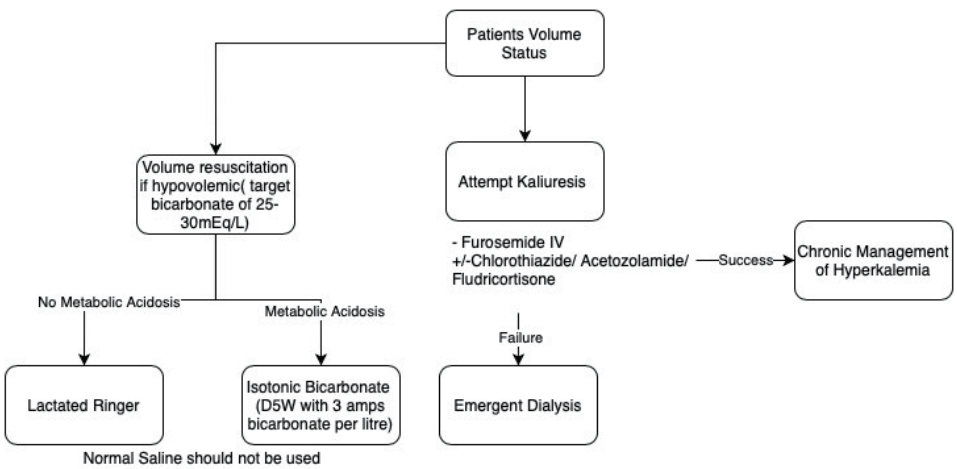


Figure 6.
Continued treatment of hyperkalemia.

- Treatment options include reviewing medication that can cause hyperkalemia, reduction in dietary potassium intake and start medication that can increase potassium excretion.

- Sodium zirconium cyclosilicate (Lokelma) should not be used for the acute management of hyperkalemia due to delayed onset of action. Onset of action is 1-6 hours with duration possibly 4-12 hours. Sodium polystyrene sulfonate (Kayexalate) has a high sodium load, and its time of onset is variable making it a poor choice for acute management.

3.2 Hypokalemia

3.2.1 Introduction

- Hypokalemia is a common electrolyte disorder defined as potassium <3.5 mEq/L (mmol/L) and can be life threatening if serum potassium <2.5 mEq/L (mmol/L). A vast majority of potassium is located intracellularly therefore hypokalemia is often due to a large total body potassium deficit [33].

- The relationship between potassium level and total body potassium deficit is exponential; as the potassium level falls progressively lower, this represents an exponentially larger decrease in the total body potassium deficit.

- Based on serum potassium, hypokalemia can be classified into:

    1. Mild Hypokalemia: - Serum Potassium 3-3.4 mEq/L (mmol/L)

    2. Moderate Hypokalemia: - Serum Potassium 2.5-2.9 mEq/L (mmol/L)

    3. Severe Hypokalemia: - Serum Potassium <2.5 mEq/L (mmol/L)

- Clinical features usually occur when serum potassium <2.5 mEq/L (mmol/L) and includes muscle pain, cramps, weakness, fatigue, constipation, syncope and palpitations [33, 34].

3.2.2 Diagnostic approach

- Hypokalemia can be caused by excessive potassium loss, inadequate intake or a transcellular shift of potassium.

- Inadequate potassium intake is a rare cause of hypokalemia and in most cases, dietary restrictions exacerbate hypokalemia due to other causes (see **Figure 7**) [33].

3.2.3 EKG changes

- EKG changes associated with hypokalemia are (see EKG 2) [33]:

    1. Decreased T wave amplitude

    2. ST-segment depression

    3. Presence of U wave (giant U waves may be mistaken for peaked T waves)

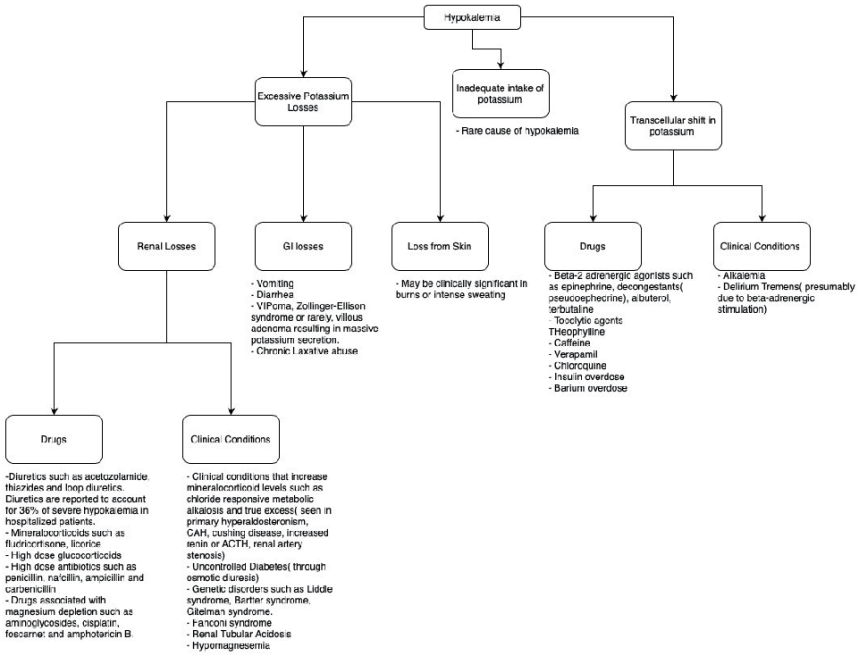
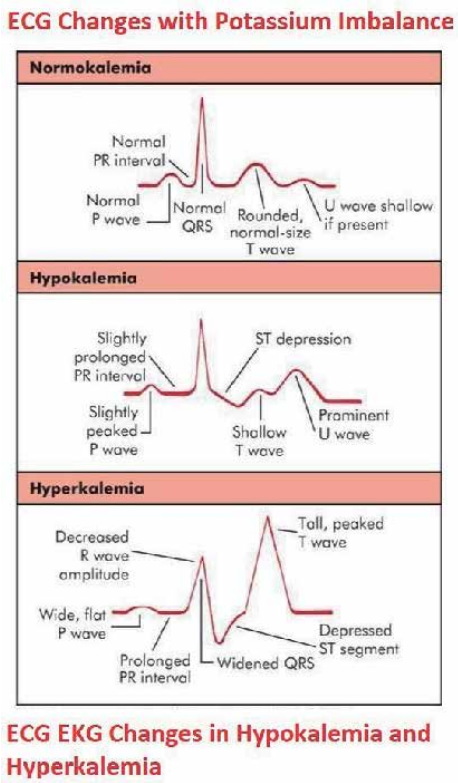


Figure 7.
Etiologies of hypokalemia.

4. Other findings include QT prolongation, ventricular extrasystoles, ventricular arrythmias.



EKG 2. EKG pattern showing changes in hypokalemia.

3.2.4 Treatment

- Goals of treatment are to reduce further potassium loss, replenish potassium stores, evaluate potential toxicities and treatment of the underlying cause [33–36].

- Due to the intracellular nature of potassium deficit means that intravascular potassium must be administered slowly, and time is required for potassium to enter the cells. Rapid administration may cause serum levels to be elevated even though there is a total body deficit leading to serum hyperkalemia.

- When treating hypokalemia, the goal potassium is >3.5 mEq/L (mmol/L). Traditionally, potassium goal >4 (mmol/L) was used to reduce the risk of arrythmias however larger studies have shown that the safest potassium level in myocardial ischemia is 3.5-4.5 (mmol/L) with evidence of higher/lower levels correlate with worse outcomes. In the specific case of DKA, with the absence of renal dysfunction, target potassium is >5.3 mEq/L (mmol/L).

- Enteral potassium repletion is preferred compared to IV route. Enteral potassium is cheaper, safer and does not irritate veins.

- Potassium chloride is the most commonly used formulation and are especially useful with metabolic alkalosis (increases serum chloride). Slow release formulations are suboptimal if immediate effect is desired however better tolerated. Another formulation is potassium citrate which may be useful in non-anion gap metabolic acidosis (the citrate will be converted into bicarbonate, thereby improving the acidosis).

- IV potassium can be used when there is lack of gut access/function, severe hypokalemia in need of emergent treatment or profound shock with severe hypokalemia. The rate of administration is 10 mEq/hour through a peripheral line or 20 mEq/hour through a central line. When IV repletion is >20 mEq/hour then continuous cardiac monitoring is suggested.

- Magnesium should be repleted as well because failure to treat this will make it difficult to fix hypokalemia. In patients with ongoing gastric losses, initiation of proton pump inhibitor may minimize electrolyte derangements.

4. Calcium and phosphate balance

- Calcium circulates in different forms. Within the plasma, 40% of calcium is bound to albumin while 15% is bound to citrate, sulfate or phosphate while 45% exists as physiologically ionized (or free) calcium. Total serum calcium is frequently misleading since it can vary based on albumin concentration and state of hydration [37].

- Plasma phosphorus exists as organic and inorganic forms. The inorganic forms are completely ionized circulating in the plasma. 99% of phosphate is present within cells.

- Only a small portion of total body calcium and phosphate is located in the plasma and it is the ionized calcium and inorganic phosphate that is regulated by hormones.

- Calcium balance is regulated by the parathyroid hormone and calcitriol which affects intestinal absorption, bone formation/ resorption and urinary excretion. Phosphorus balance is primarily regulated by the parathyroid hormone [38].

- Most of the body's calcium as well as phosphate exists in bone which functions as a reservoir to maintain normal plasma ionized calcium and phosphate concentration.

- Ionized calcium is the best measurement of biologically active calcium in critically ill patients.

4.1 Hypocalcemia

- Common electrolyte abnormality defined as ionized calcium level < 4.4 mg/dl or total calcium level < 8.4 mg/dl (corrected by albumin). Critically ill patients are commonly affected. Ionized calcium <0.65 is critically low which could cause hypotension.

4.1.1 Clinical presentation

- Clinical presentation of hypocalcemia can vary from asymptomatic to severe symptoms. Acute hypocalcemia can present with neuromuscular irritability (with numbness/tingling of perioral region, fingers/toes), myalgias, muscle cramps/spasms or tetany.

- Chronic hypocalcemia (develops over years) is often asymptomatic however still possible to cause neuromuscular irritability.

- Trousseaus sign is a hallmark sign of acute hypocalcemia in which 94% of patients will have a positive sign [39].

- EKG changes that are seen in hypocalcemia are

    1. QT segment prolongation or ST segment lengthening

    2. AV conduction block

    3. Acute anteroseptal injury without myocardial infarction

    4. Abnormal T waves

4.1.2 Diagnostic approach

- When considering treating hypocalcemia, always confirm with ionized calcium, magnesium and phosphate. Magnesium abnormalities can lead to functional hypoparathyroidism.

- Calcium should also be corrected based on serum albumin.

- Various causes of hypocalcemia are

    1. Medications: - Anticonvulsants (phenytoin, phenobarbital, carbamazepine), antibiotics (rifampin, aminoglycosides, foscarnet), loop

diuretics, chemotherapy (cisplatin, 5-fluorouracil) and drugs that inhibit bone reabsorption (bisphosphonates, calcitonin and denosumab)

2. Severe Inflammation seen in sepsis or major buns

3. Pancreatitis (especially in hypertriglyceridemia induced pancreatitis)

4. Increased Citrate:- Seen in massive transfusion, plasmapheresis, leukapheresis and renal replacement therapy.

5. Alkalosis

6. Chronic conditions such as hypoparathyroidism, Vit D deficiency or osteoblastic metastasis.

7. Chronic Kidney disease (most common cause)

- Most critically ill patients have hypocalcemia and treatment is usually not indicated. Treatment is indicated when patient is symptomatic, presence of prolonged QT interval or severe hypocalcemia (ionized calcium < 0.8) [37, 39].

- IV calcium can be used in symptomatic/severe cases or in the presence of EKG changes. IV formulations are calcium chloride (central access) and calcium gluconate (peripheral access). Both are equally fast however calcium chloride can cause tissue necrosis if it extravasates.

- First, IV loading dose can be given (1 g calcium chloride or 2-3 g calcium gluconate) followed by maintenance doses if there is an ongoing process with smaller doses (for example calcium gluconate 1 g q1h as needed). IV calcium increases ionized calcium in patients with hypocalcemia, but randomized trials have not evaluated effect on clinical outcomes [40]. IV calcium can eventually be transitioned to oral formulations.

- For mild- moderate hypocalcemia, therapy can be started with oral calcium. Usual dose is calcium carbonate 1 g every 12 hours.

- Treatment of hypocalcemia is contraindicated in hyperphosphatemia (could cause precipitation of calcium phosphate, calciphylaxis), ethylene glycol poisoning (calcium promotes calcium oxalate precipitation in the brain) and digoxin poisoning (theoretical contraindication).

4.2 Hypercalcemia

4.2.1 Introduction

- Hypercalcemia is a serum calcium>10.5 mg/dl or ionized calcium>5.6 mg/dl. Calcium is partially bound to albumin therefore should be adjusted based on albumin. Only ionized calcium is biologically active so, if available, ionized calcium should be used to manage hypercalcemia among critically ill [41].

- Hypercalcemia can be classified based on severity:

1. Mild Hypercalcemia:- Total calcium 10.5-12 mg/dl or ionized calcium 5.6-8 mg/dl

2. Moderate Hypercalcemia:- Total calcium 12-14 mg/dl or ionized calcium 8-10 mg/dl

3. Hypercalcemic Crises:- Total calcium >14 mg/dl or ionized calcium >10 mg/dl.

4.2.2 Clinical features

- Mild hypercalcemia may be asymptomatic however rapid increases are more likely is be associated with symptoms than chronic hypercalcemia.

- This can present as bone pain, delirium (which could progress to stupor/coma), paresthesia, muscle weakness, GI symptoms (abdominal pain, pancreatitis, constipation, ileus, nausea/vomiting).

- Hypercalcemia does not commonly affect EKG or cardiac function however short QT interval may be a common finding.

4.2.3 Diagnostic approach

- Various causes of hypercalcemia are:

1. Hyperparathyroidism (Primary and tertiary)

2. Malignancy: Approximately 80% of these cases are caused by increased PTH-related peptide (most often squamous cell carcinoma of lung/head/neck) which is a protein that mimics PTH. 20% of cases is due to direct bone invasion.

3. Medications: - Vit A/D excess, increased calcium intake (milk-alkali syndrome, chronic renal failure), teriparatide, lithium, thiazide diuretics, TPN

4. Granulomatous diseases:- Such as sarcoidosis, TB, fungal infections

5. Rhabdomyolysis

6. Addison's disease

7. Paget's disease

4.2.4 Treatment

- Overall, almost 90% of hypercalcemia is due to primary hyperparathyroidism or malignancy.

- Factitious Hypercalcemia may also occur when total calcium is elevated but ionized calcium is normal. This occurs when serum albumin or protein levels are elevated.

- Initial volume resuscitation is essential since hypercalcemia typically causes volume depletion due to enhanced fluid excretion by the kidneys and reduced oral intake. Plasmalyte is a good choice since it is a balanced crystalloid which does not contain calcium. Lactated ringer contains calcium and normal saline can cause acidosis (possibly increasing risk of renal injury) therefore both are suboptimal compared to plasmalyte (see **Figure 8**).

- Mild to moderate hypercalcemia without symptoms does not require aggressive treatment. The underlying disease should be treated, and potentially contributing medication discontinued. Immobility may exacerbate hypercalcemia therefore patients should be mobilized.

- In patients with severe hypercalcemia, IV fluid hydration (at least 2-4 L/day for 1-3 days) should be given in association with bisphosphonates and calcitonin to reduce serum calcium levels.

- Bisphosphonates block calcium release from bones causing unidirectional uptake by the bones. These take days to work and should be started early. Bisphosphonates should be avoided in patients with increased calcium intake (milk-alkali syndrome. The main side effect is renal failure however the most common is flu-like syndrome which can be treated symptomatically. Various options are pamidronate 60-90 mg IV or zoledronic acid 4 mg IV [41, 42].

- Calcitonin is an excellent agent to control severe symptomatic hypocalcemia while waiting for bisphosphonates to take effect. These work by reducing bone calcium reabsorption and cause a temporary reduction in calcium. Calcitonin can cause nausea, vomiting and flushing. For adults, calcitonin 4 U/kg

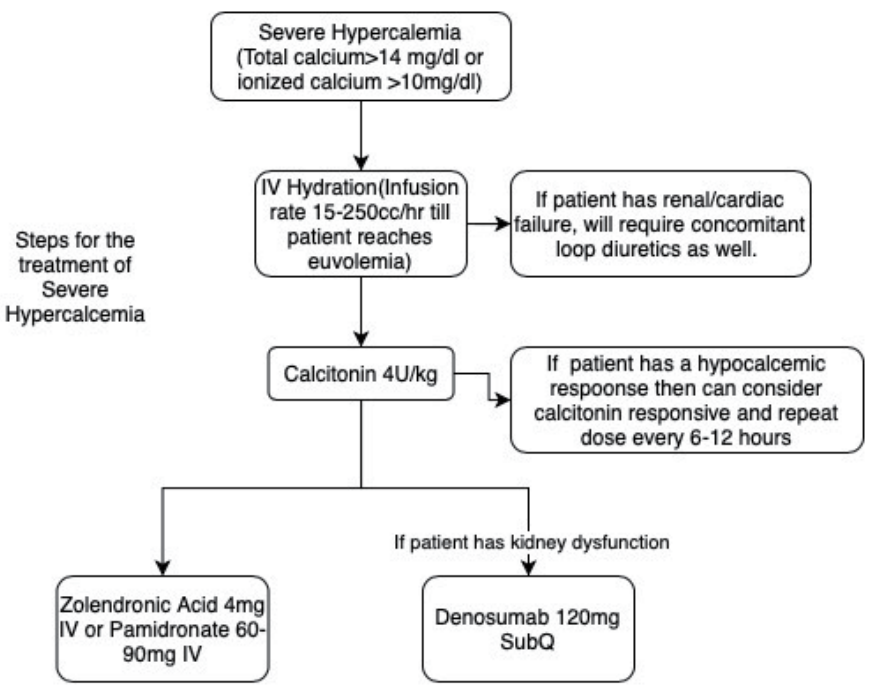


Figure 8.
Treatment of hypercalcemia.

subcutaneously every 12 hours for 24 hours; effect of calcitonin is short lived, and tolerance typically develops within 2 days [43].

- In patients who bisphosphonates and calcitonin are ineffective, denosumab (monoclonal antibody that inhibits osteoclast formation and bone resorption) can be considered.

- Loop diuretics can be used once volume status normalizes to enhance calcium excretion and to avoid volume overload. They may have to be started earlier if patient has a history of congestive heart failure or kidney disease. Glucocorticoids can be given in patients with granulomatous disease, Vit D overdose or malignancy to inhibit conversion of Vit D to calcitriol.

- In renal failure, dialysis with low calcium bath is an option.

4.3 Hyperphosphatemia

- Hyperphosphatemia is defined as serum phosphate >4.5 mg/dl in adults.

- Hyperphosphatemia is itself, asymptomatic however can indirectly cause symptoms by causing symptomatic hypocalcemia (by binding to calcium) or calciphylaxis (precipitation of calcium phosphate in tissues which can manifest as skin ulceration).

- Sustained hyperphosphatemia generally occurs in renal failure since normally the kidneys are efficient in phosphate excretion. Possible inciting events are [44]

    1. Tissue necrosis: Tumor lysis syndrome, rhabdomyolysis, hemolysis, fulminant hepatitis, severe hyperthermia

    2. Endocrinopathy: Hypoparathyroidism, hypothyroidism/ hyperthyroidism, adrenal insufficiency, acromegaly

    3. Medications: Exogenous phosphate intake (phosphate containing laxatives/enemas, TPN), Vit D toxicity, bisphosphonates, fosphenytoin

- False elevation of phosphate can be seen in hyperlipidemia, hyperbilirubinemia, hyperglobulinemia (multiple myeloma) or a hemolyzed specimen.

- Calcium phosphate product (see Eq. 4) can predict the risk of calciphylaxis and is more important than the phosphate level alone. Calcium-phosphate product>70 causes an increased risk of calciphylaxis.

$$Calcium\ Phosphate\ Product = Serum\ Calcium\ x\ Serum\ Phosphate \quad (4)$$

- Acute treatment of hyperphosphatemia (see **Figure 9**) includes treating inciting event, phosphate restricted diet, fluid resuscitation and forced diuresis (acetazolamide+ − furosemide) or dialysis [44].

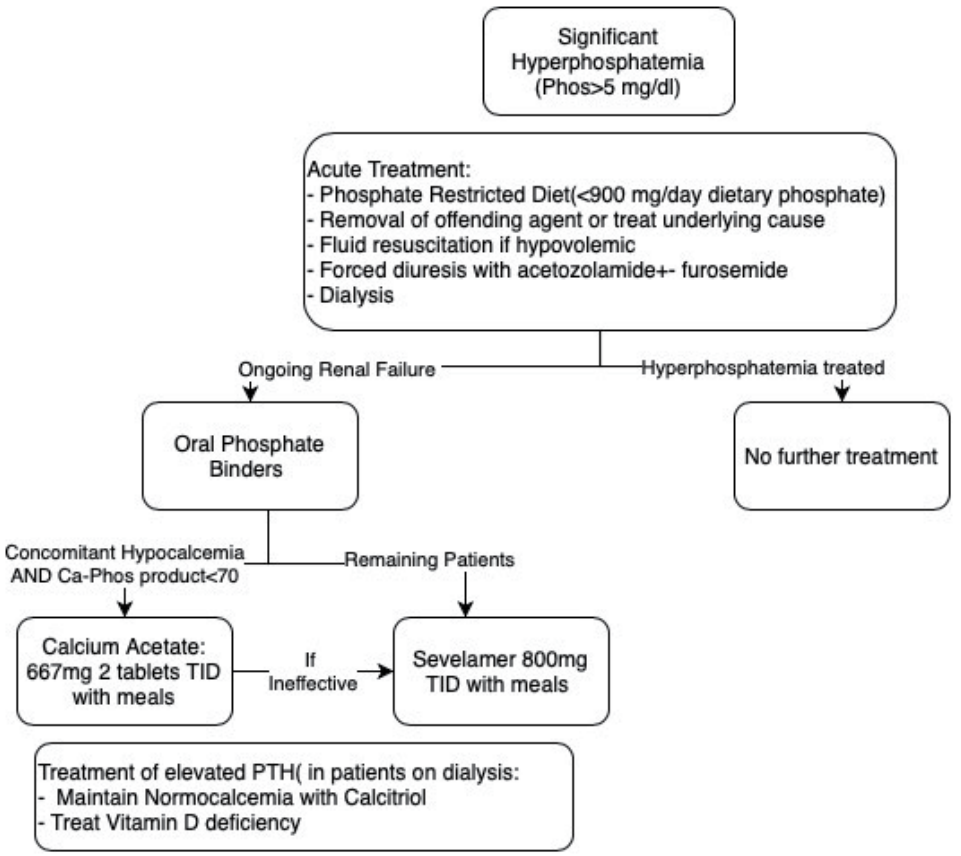


Figure 9.
Treatment of hyperphosphatemia.

- If patient has persistent renal failure, can start oral phosphate binder. Calcium acetate can be useful in patients with concomitant hypocalcemia and should be avoided in patients with hypercalcemia, Vitamin D toxicity and Ca-Phos product>66. Sevelamer is a nonabsorbable resin that is preferred for patients on dialysis [44].

4.4 Hypophosphatemia

- Hypophosphatemia is defined as serum phosphate <2.5 mg/dl.

- Patients with hypophosphatemia can present with paresthesia, tremors, seizures, impaired heart contractility, arrythmias, muscle weakness (including the diaphragm). Usually, symptoms occur at levels <1-2.5 mg/dl. Most cases of mild (or even moderate) hypophosphatemia are asymptomatic.

- Causes of hypophosphatemia are [45]:

    1. Shifting phosphate into cells: Diabetic ketoacidosis, refeeding syndrome, respiratory alkalosis, hungry bone syndrome

2. Reduced GI uptake: Inadequate oral intake, chronic diarrhea, drugs (chronic use of antacids containing calcium, magnesium or aluminum).

3. Increased Renal losses: Diuretics (loop diuretics, acetazolamide, thiazides), osmotic diuresis, auto-diuresis (post-ATN, iatrogenic volume overload), CRRT, hyperparathyroidism, other medications (aminoglycosides, IV iron, tenofovir, chemotherapeutic agents).

4. Multifactorial: Alcoholism, Vitamin D deficiency, critical illness (sepsis, trauma, major surgery, burns).

5. Potential causes of pseudo-hypophosphatemia are hyperbilirubinemia, mannitol, paraproteins and acute leukemia.

- Generally, hypophosphatemia can be determined by history and review of labs/medication. Fractional excretion of phosphate can be helpful in cases when cause is unclear. Fractional excretion of phosphate should be <5% as a normal response to hypophosphatemia however >5% can be seen in renal phosphate wasting [45, 46].

- IV phosphate can be given for severe hypophosphatemia, symptomatic, or in patients with lack of enteral access or malabsorption. These should be infused slowly since rapid infusion can lead to transient hyperphosphatemia (leading to hypocalcemia). Either potassium or sodium phosphate can be used.

- Oral phosphate can be given however tends to cause diarrhea. It is available as Phos-NAK packets (which contains 8 mM phosphate, 7 mEq potassium and 7 mEq sodium), oral sodium phosphate liquid and oral potassium phosphate liquid [47].

    - Phosphate ≤1.5 mg/dl:- Orally, 16 mM phosphate every 6 hours. Intravenously, initial dose can be 30 mM infused over 4 hours

    - Phosphate >1.5 mg/dl:- Orally, 8 mM phosphate every 8 hours. Intravenously, initial dose of 15 mM phosphate can be infused over 2 hours.

- Patients with active refeeding syndrome and morbid obesity, can consider using higher doses than indicated based on phosphate levels.

5. Magnesium homeostasis

- Magnesium is the 4th most abundant cation in the body. Magnesium homeostasis needs to be tightly regulated and thus facilitated by intestinal absorption and renal excretion. Magnesium plays an essential role in bone formation, neuromuscular stability and muscle contraction.

5.1 Hypomagnesemia

- Magnesium <1.8 mg/dl is defined as hypomagnesemia. Reported prevalence of hypomagnesemia ranges from 2.5-15% in the general population however higher in the ICU setting.

- Most patients are asymptomatic until concentration is <1.2 mg/dl however presentation can overlap with other abnormalities. Patients can present with nausea/vomiting, loss of appetite, neuromuscular irritability, tremors/tetany, hypocalcemia, hypokalemia, seizures, psychosis and arrythmias [48].

- Hypomagnesemia induced EKG changes include:

    1. Flattened T wave and U waves

    2. Prolonged QT interval and widened QRS complex

    3. Prolonged QT interval

- Causes of hypomagnesemia are:

    1. Medications: - Diuretics (except potassium sparing diuretics), antibiotics such as aminoglycoside, amphotericin and pentamidine, cyclosporine and tacrolimus, platinum based chemotherapy and proton pump inhibitors

    2. Hypercalcemia, hyperphosphatemia, metabolic acidosis

    3. Renal disease: - Post-ATN diuresis, osmotic diuresis, renal tubular acidosis

    4. GI Losses: - Malabsorption, diarrhea/vomiting, pancreatitis

    5. Chronic alcoholism, diabetes, large volume transfusion of citrated blood products, sepsis

- Magnesium repletion is generally safe except for myasthenia gravis (due to increased risk of muscle weakness) and renal failure.

- For patients with mild hypomagnesemia (1.5-2 mg/dl), oral magnesium can be used. Oral formulations are magnesium oxide 400 mg twice a day or magnesium hydroxide milk of magnesia) 15 ml once daily. If unable to take PO medication, 2 g of IV magnesium sulphate can be given.

- For moderate hypomagnesemia (1.2-1.5 mg/dl), intermittent infusions of 2-4 g magnesium sulphate IV can be given. To improve intracellular absorption, the dose can be infused for a longer period of time.

- For severe hypomagnesemia (<1.2 mg/dl), multiple doses of IV magnesium can be given or a continuous infusion of IV magnesium (4-8 g IV magnesium sulphate over 24 hours) (see **Figure 10**).

- 1 g magnesium sulphate is equivalent to 100 mg of elemental magnesium.

- In Torsade de Pointes or seizures secondary to hypomagnesemia, patients can be loaded with 2 g magnesium sulphate over 5-15 min followed by 2 g additionally over 30-60 min. These are followed by a continuous infusion of magnesium sulphate 1 g/hour. If the magnesium level is 5-7 mg/dl, the infusion should be reduced by 50%. If magnesium is >7 mg/dl then the infusion should be stopped.

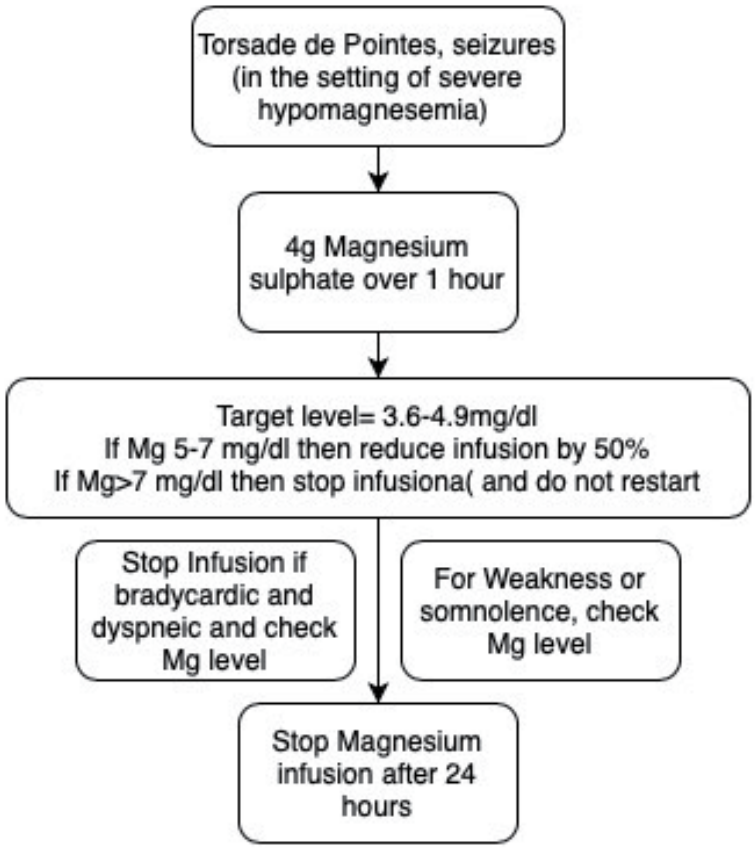


Figure 10.
Treatment of severe symptomatic hypomagnesemia.

5.2 Hypermagnesemia

- Magnesium>2.6 mg/dl is defined as hypermagnesemia. Patients with serum magnesium<4.8 mg/dl are usually asymptomatic, deep tendon reflexes may be diminished with serum magnesium>6.1 mg/dl and absent when >12 mg/dl [48].

- Patients can present with lethargy, confusion, nausea, vomiting, bradycardia. In severe cases, muscle weakness, respiratory distress, apnea, heart block, severe bradycardia, delirium and coma [48].

- EKG will show widened QRS complex with peaked T waves. Heart block can also be seen.

- Renal failure is required in addition to another source of magnesium to cause persistent hypermagnesemia. Concomitant causes of hypermagnesemia are:

    1. Exogenous magnesium:- Magnesium infusion for pre-eclampsia, magnesium containing antacids and magnesium containing laxatives/enema

    2. Endogenous magnesium from cellular lysis:- Rhabdomyolysis, hemolysis, tumor lysis syndrome and crush injury, severe burns.

- In most cases of hypermagnesemia, discontinuing magnesium containing drugs or supplements or volume replacement can sufficiently treat it [48].

- In patients with moderate hypermagnesemia (3.6-10 mg/dl or no cardiac/respiratory symptoms), the underlying cause should be treated. Furosemide can be used to enhance magnesium excretion.

- In patients with severe hypermagnesemia (causing cardiac/respiratory symptoms), IV calcium is required to stabilize the myocardium (2 g of calcium gluconate IV over 5-10 min followed by a continuous infusion in severe cases). In patients who are non-oliguric, furosemide with IV fluids can be used for elimination of magnesium. In patients who are oliguric, emergent dialysis is required.

6. Common conditions in the ICU

6.1 Massive transfusion protocol

- Massive transfusion protocol should be used in critically ill bleeding patients anticipated to require massive transfusion.

- Two common electrolytes that occur during MTP are hypocalcemia and hyperkalemia.

- Hypocalcemia is caused by the presence of the anticoagulant citrate (each bag on pRBC contains 3 g citrate). Normally, this amount can be rapidly cleared by the liver however in critically ill patients receiving multiple units, the process of liver elimination is compromised. Citrate accumulates in the blood where it binds to circulating ionized calcium thereby causing hypocalcemia [49].

- Bedside measurement of calcium can be used to guide calcium management. When administering MTP (around 6 units pRBC), it is reasonable to administer 3 g calcium gluconate.

- Hyperkalemia has been shown to be a risk when patients are transfused >7 units of pRBC [50]. This can be exacerbated in patients with renal failure, effective circulating volume depletion or more commonly hypoaldosteronism. There have been studies suggesting there may be a link between incidence of hyperkalemia and the use of washed or unwashed blood products and length of RBC storage [51].

Abbreviations

ICU	Intensive Care Unit
RAAS	Renin Angiotensin Aldosterone System
mEQ/L	milli-equivalent per liter
mMol/L	milli-mole per liter
V2 receptors	Vasopresin 2 receptors
NaCl	Sodium Chloride
Lab	Laboratory
ADH	Anti-Diuretic Hormone

Mosmol	Milli-osmols
EABV	effective arterial blood volume
SIADH	syndrome of inappropriate anti-diuretic hormone
Mcg	microgram
SubQ	subcutaneous
D5W	5% dextrose solution in water
MRI	magnetic resonance imaging
DWI	diffusion weighted imaging
EKG	electrocardiogram
AKI	acute kidney injury
ESRD	End-stage renal disease
DKA	diabetic ketoacidosis
IV	intravenous
Mg	milligram
Dl	deciliter
Vit D	Vitamin D
TPN	total parenteral nutrition
PTH	parathyroid hormone
GI	gastrointestinal
ATN	acute tubular necrosis
CRRT	continuous renal replacement therapy
PO	per-oral
MTP	massive transfusion protocol

Author details

Syed Zaidi[1]*, Rahul Bollam[1] and Kainat Saleem[2]

1 Department of General Internal Medicine, UPMC Mercy, Pittsburgh, United States of America

2 Department of General Internal Medicine, UPMC PUH/SHY, Pittsburgh, United States of America

*Address all correspondence to: zaidi_arsalan@hotmail.com

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Metals, inorganic compounds, and their elements act as cofactors for enzymes that play an essential role in various human biological processes. These mineral nutrients come from the soil and enter the human body through the food chain via plants. A regulated diet with all necessary constituents consumed in an appropriate way maintains cell homeostasis and keeps the body under a physiological state essential for cellular demands. This book deals with problems of mineral deficiencies, which can arise due to decreased consumption of certain foods, malabsorption syndrome, bleeding disorders, a diet with insufficient nutritional content, and so on.

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