Advances in Health and Disease

Advances in Health and Disease

Volume 25

Lowell T. Duncan
Editor

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ADVANCES IN HEALTH AND DISEASE

VOLUME 25

ADVANCES IN HEALTH AND DISEASE

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ADVANCES IN HEALTH AND DISEASE

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VOLUME 25

LOWELL T. DUNCAN
EDITOR

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NOTICE TO THE READER

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PREFACE

Advances in Health and Disease. Volume 25 first offers nurses, emergency-care doctors, psychiatrists, and other personnel involved in the multidisciplinary assessment, management and treatment of intoxicated patients in the emergency department setting an overview of the literature on psychomotor agitation associated with alcohol or drug abuse.

Next, this compilation explores the commonly neglected aspects of oral health in school children, such as dental caries, pain, dental trauma, developmental anomalies and malocclusion.

The authors discuss hemolysis in blood samples, a problem that is commonly found in laboratories. This has a potential impact on the quality of blood tests, completion time or turn around time, and creates discomfort for patients due to repeated sampling.

The available research on the effect of yoga in managing the "ominous octet" of type 2 diabetes is summarized in an effort to ensure the evidence-based practice of therapeutic yoga in a clinical setting.

The effects of age and gender on cystatin C levels are investigated, challenging the glomerular filtration rate equations for healthy cases.

The closing study explores how deficiencies such as zinc, folic acid and vitamin B12, as well as an immune deficiency syndromes, may cause a sprue-like enteropathy.

Chapter 1 - Altered mental status, psychomotor agitation and aggressive behaviours are important causes of Emergency Department (ED) admission, especially when alcohol or drugs intoxication/withdrawal are concomitant. The ED setting plays an important role in assessment, management and treatment of acute agitation in intoxicated patients, which often determines potential risk of morbidity or death if not diagnosed and managed, through the rapid control of the state of agitation. The treatment of agitation episodes may represent risks both for patients and healthcare professionals. The objective of the present chapter is to offer to nurses, emergency-care doctors, psychiatrist, and other personnel involved in the multidisciplinary assessment, management and treatment of intoxicated patients in the ED setting an overview of the literature on psychomotor agitation associated to alcohol or drug abuse. This was made possible by the review of current literature, identifying study designs and sampling features, as well as the measures used to determine the level of agitation, metabolic or neurological parameters alterations in alcohol or drug use, summarizing the findings on prevalence estimates for different drugs used among ED patients.

Chapter 2 - The oral health-related quality of life (OHRQoL) has been defined as a "multidimensional construct that includes a subjective evaluation of individual's oral health, functional well-being, emotional well-being, expectations and satisfaction with care, and sense of self" [1]. In this chapter, the common oral health-related neglected aspects in school children like- dental caries, pain, dental trauma, developmental anomalies and malocclusion will be discussed including the overall impact on their daily life on the basis of academic performance, physical performance, socioemotional development. In order to address and counteract these circumstances at the primitive stage, the contemporary preventive oral health care measures and dental interventions will also be discussed. This will enlighten the parents, school teachers, nurses, coaches and oral health professionals about the basic oral health needs of school children for the overall improvement of their quality of life.

Chapter 3 - Hemolysis in blood samples is a problem that is commonly found in laboratories. This has a potential effect on the qualiy of blood tests, completion time or TAT (turn around time), and creates discomfort for

patients due to repeated sampling. Hemolysis can occur in vitro or in vivo. In vitro hemolysis can occur due to lysis of red blood cells during the sample collection and handling of blood samples. In vitro hemolysis is a result of pre-analytical causes associated with sample collection, jarring transportation methods, extreme temperature, sample handling, delayed processing, and prolonged storage. In vivo hemolysis occurs if the rate of erythrocyte damage increases, thereby reducing the life span of erythrocytes. Hemolysis results in a decrease in the number of RBCs and HCT values due to lysis. Hematological instruments usually lyse the sample before measuring the HGB, the number of PLT, the number of WBC, and the number of WBC differential cells, so these values are usually not affected by hemolysis. In samples with hemolysis, the ESR values will decrease. In the ESR test with the Westergreen method, it is often found that hemolysis samples are difficult to assess because of unclear boundaries. The mechanism for shortening APTT in hemolysis samples has not been confirmed. This is thought to be caused by the release of phospholipids from erythrocytes and intracellular substances from leukocytes and platelets which can activate Cascade coagulation. Other literature states that activating the freezing cascade will cause PPT shortening and decreased fibrinogen levels, whereas APTT can extend or shorten the levels depending on whether there is activation or loss of fibrinogen. Hemolysis samples that are immediately examined may experience coagulation activation, so the APTT results are shortened.

Chapter 4 - Type 2 diabetes (T2DM) is a chronic metabolic disorder which significantly impacts health, quality of life and life expectancy. From triumvirate model to the egregious eleven model, our understanding on the pathophysiology of type 2 diabetes is ever expanding. The chronicity of diabetes mellitus, the involved direct and indirect costs, and adverse effects associated with the conventional medicines make increasing number of patients turn towards complementary and alternate (CAM) therapies. Yoga is one such CAM therapy which is classified under mind-body medicine by the world health organisation (WHO). The significant role played by physical exercise in the effective management and prevention of T2DM is well documented. The available scientific literature on the benefits of yoga

clearly demonstrates that yoga is more than just a milder form of physical exercise. Beneficial effects of yoga, especially in reducing inflammation, oxidative stress, salivary cortisol, lipid profile, autonomic imbalance and glycemic control has been well documented. Inflammation and oxidative stress plays a central role in the underlying pathophysiology of diabetes (both insulin resistance & beta cell dysfunction) and its complications. One distinguishable advantage of using yoga over physical exercises is the relatively lower cardiovascular demand which makes yoga. Apart from the common pathophysiological abnormality of insulin resistance (in muscles and liver) and beta cell dysfunction, the 'Ominous octet' model of DeFronzo refers to the involvement of brain, adipose tissues, gastrointestinal hormones, kidney and alpha cells in the pathophysiology of T2DM which requires multiple drug combination to rectify the underlying pathophysiological abnormalities, instead of simply trying to reduce the HbA1C levels. The current review is aimed at summarising the available research evidences on the effect of yoga in managing the 'Ominous octet' of T2DM and ensure evidence-based practise of therapeutic yoga in a clinical set up.

Chapter 5 - Background: Cystatin C has gained more attention as a promising biomarker due to several advantages over creatinine that suffers from the blind range (does not increase until 50% of the kidney deteriorates). Cystatin C levels are influenced as soon as any mild defect in the kidney occurs. Several non-renal diseases influence cystatin C. Thus, providing additional prognostic value for this promising biomarker. Objectives: 1. Investigate the effects of age and gender on cystatin C levels. 2. Challenge the glomerular filtration rate equations for healthy cases. 3. Compare the values obtained from different glomerular filtration rate equations. 4. Evaluate the prognostic value of cystatin C for selected non-renal diseases. Methods: Using cross-sectional analyses, the authors established the relationship between cystatin C levels and non-renal predictors. The quantification of cystatin C was performed by high-performance liquid chromatographic method, while for creatinine by a colorimetric enzymatic method. Results: Statistical data confirmed a non-significant relationship concerning age, gender, or smokers among the recruited healthy samples.

For the recruited patients suffering from diabetes, hyper- and hypothyroidism, and cardiac dysfunctions, an apparent increase in cystatin C levels was observed except for hypothyroidism patients in which a decrease in their cystatin C levels was observed. Conclusion: Diabetes, thyroid, and cardiac dysfunctions showed to influence the levels of cystatin C in human blood. On the other hand, age, gender, and smoking habit didn't show to influence cystatin C levels. Therefore, cystatin C could be considered as a useful biomarker of the mentioned diseases, in turn, this requires extra precautions including the evaluation of several clinical conditions by physicians should cystatin C is considered as a renal biomarker.

Chapter 6 - Celiac disease (also termed gluten-sensitive enteropathy or celiac sprue) is a gluten-dependent immune-mediated disorder of the small intestine that occurs in genetically-predisposed persons. Serological studies have estimated that about 1% of screened individuals, possibly more, have celiac disease. The precise precipitating event leading to clinical illness is not known. A number of disorders may have the pathological appearances of celiac disease, such as mucosal injury from oats, other proteins such as soy and a wide array of infections, including protozoans, viral, bacterial and parasitic agents. Some deficiencies including zinc, folic acid and vitamin B12 as well as an immune deficiency syndromes may cause a sprue-like enteropathy. In recent years, medications including pharmacological and biological agents have been recognized ranging from drugs like olmesartan for hypertension to an emerging group of biological agents, particularly checkpoint inhibitors for advanced malignancies.

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Chapter 1

MANAGEMENT OF PSYCHOMOTOR AGITATION ASSOCIATED WITH ALCOHOL AND/OR DRUGS INTOXICATION/WITHDRAWAL IN THE EMERGENCY DEPARTMENT

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ABSTRACT

Altered mental status, psychomotor agitation and aggressive behaviours are important causes of Emergency Department (ED) admission, especially when alcohol or drugs intoxication/withdrawal are concomitant. The ED setting plays an important role in assessment, management and treatment of acute agitation in intoxicated patients, which often determines potential risk of morbidity or death if not diagnosed and managed, through the rapid control of the state of agitation. The treatment of agitation episodes may represent risks both for patients and healthcare professionals.

The objective of the present chapter is to offer to nurses, emergency-care doctors, psychiatrist, and other personnel involved in the multidisciplinary assessment, management and treatment of intoxicated patients in the ED setting an overview of the literature on psychomotor agitation associated to alcohol or drug abuse. This was made possible by the review of current literature, identifying study designs and sampling features, as well as the measures used to determine the level of agitation, metabolic or neurological parameters alterations in alcohol or drug use, summarizing the findings on prevalence estimates for different drugs used among ED patients.

INTRODUCTION

Altered mental status, which includes the undifferentiated presentation of disorders of mentation (impaired cognition, diminished attention, reduced awareness, and/or altered level of consciousness), is an important cause of Emergency Department (ED) admission (4-10% of all the patients), frequently as a concurrent issue in patients with other primary presentations (Kanich et al. 2002). One of the most serious difficulties, in the context of ED setting, is represented by acute agitation, which often hides serious diseases, with the consequent potential risk of morbidity or death if not diagnosed and managed, through the rapid control of the state of agitation (Gottlieb, Long, and Koyfman 2018a).

In a recent study, Nordstrom and coworkers (Nordstrom et al. 2012) have defined agitation as a "temporary disruption of the typical physician-patient collaboration which has unintended consequences for the staff or

other patients." It is a common knowledge that treatment of agitation episodes may lead to potential harm among patients and may represent risks for healthcare professionals (Gates et al., 2006). When de-escalation attempts fail, ED staff may be forced to use physical force (Wong et al. 2020). Therefore it seems necessary during agitation management to analyze how to early detect and to prevent escalating agitation, minimizing the use of restraints (Knox and Holloman 2012).

Mounting evidence has shown that acute agitation has various etiologies, including medical (hypoglycemia, pain, delirium), substance-induced (alcohol or drugs), and psychiatric causes (Justice 2012; Wilson, Pepper et al. 2012; Nordstrom et al. 2012; Choo, 2016). Excited delirium or excited delirium syndrome (ExDS) represents a typical feature for a large number of patients with acute agitation who come to the ED (Gonin et al. 2017; Stowell et al. 2012).

In the literature, ExDS is described as an extremely violent condition, characterized by incoercible psychomotor agitation and aggressiveness (Vilke et al. 2012) which requires the use of physical restraint and which includes the intervention of law enforcement officers. Even if a standardized definition of ExDS diagnostic criteria doesn't exist, since 2009 the American College of Emergency Physicians (ACEP) has defined a minimum of six criteria to diagnose ExDS which includes acute delirium (not linked to dementia or preexisting pathologies) associated with extreme physical and psychomotor agitation (Salem, Carolina, and Chan 2009).

Causes of Agitation (Kanich et al. 2002))

The agitation of the patient that can be observed in the ED has a wide variety of presentation and different primary causes.

Primary Psychosis or Bipolar Disorders

they are medical emergencies frequently treated in the ED (Zun and Downey 2008). Individuals affected by schizophrenia or bipolar disorder, especially during the relapse or exacerbation phases of the disease, may

present excessive verbal and motor behaviour, becoming vulnerable to episodes of agitation (Zeller and Citrome 2016). Agitation presented by patients with psychiatric disorders requires immediate action to prevent escalation, preferably based on the support offered by a psychiatrist or social worker when available, otherwise by emergency physicians. Early interventions are very important, especially for younger, first-episode psychotic patients. Mental health and emergency care collaboration is warranted to achieve this goal (Peltzer-Jones et al. 2019).

Secondary to Severe Diseases

- Neurologic diseases such as a direct central nervous system (CNS) process (stroke, head trauma, dementia, encephalitis or mass lesion) or a secondary event with neurologic impact on CNS (metabolic/endocrine, sepsis, cardiogenic shock, or intoxication by medications or recreational drugs) (Mantovani et al. 2010; Banno et al. 2014).
- Systemic diseases affecting metabolic/endocrine system (electrolyte abnormalities, hypoglycemia, hyperglycemia, hypoxia, hypercarbia, renal or liver failure, thyrotoxicosis myxedema coma, nutritional deficiency, infection, sepsis, systemic infections, fever-related delirium) or other conditions (such as shock, burn, hypothermia, hyperthermia).

When a neurological or medical condition is strongly suspected, agitated patients who come to ED have abnormal vital signs. A medical workup, including laboratory analysis, neuroimaging, or lumbar puncture, has to be targeted to the underlining medical condition (Nordstrom et al. 2012).

Recreational Drugs

Alcohol Intoxication and Withdrawal (Caputo et al. 2019):

Alcohol consumption is responsible respectively for 5.9% of all causes of death and 5.1% of all causes of disease, with a worldwide consumption

spread of alcoholic beverages of 2 billion people consume (World Health Organisation 2014). The new version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) has introduced the Alcohol Use Disorder (AUD) to describe the chronic use of alcohol (NIAAA 2013). The prevalence of AUD in most Western societies ranges from 13 to 30% with a 2:1 ratio in men and women (World Health Organisation 2014; Grant et al. 2015). Patients with AUD are often admitted to general hospitals, especially to hospital intensive care units (De Wit et al. 2010) presenting conditions of acute alcohol intoxication (AAI) or alcohol withdrawal syndrome (AWS) which require a specific treatment approach. AWS appears when an alcoholic reduces or discontinues alcohol consumption; in 3–5% of these individuals, grand mal convulsions, severe confusion, delirium tremens, or both develop (Schuckit 2014). AAI is an intercurrent, potentially transitory condition that occurs as a result of excessive alcohol intake, in which the rapid crossing of the blood-brain barrier by large quantities of alcohol is initially responsible for behavioural alterations such as euphoria, dysphoria, social disinhibition, sleepiness, belligerence and aggressiveness, and, as the blood alcohol concentration increases, lethargy, stupor and coma can gradually appear (Schuckit 2006; Vonghia et al. 2008). A transitory memory deficit may occur and the subject may not remember what happened before the alcohol consumption (Schuckit 2006; Vonghia et al. 2008). AAI may cause several metabolic alterations (hypoglycaemia, lactic acidosis, hypokalaemia, hypomagnesaemia, hypoalbuminaemia, hypocalcaemia, and hypophosphataemia), may lead to neurological complications (myopathy, muscular flaccidity and rhabdomyolysis, with potential death due to the onset of hyperkalaemia and acute renal insufficiency), cardiovascular effects (tachycardia, peripheral vasodilatation, and volume depletion, "holiday heart syndrome," characterized by atrial or ventricular tachy-dysrhythmias and new-onset atrial fibrillation after acute alcohol ingestion) (M A Schuckit 2006; Vonghia et al. 2008), gastrointestinal effects (nausea, vomiting, diarrhea, abdominal pain secondary to gastritis, peptic ulcer, and pancreatitis, acute alcoholic hepatitis (AAH), associated with alcoholic liver cirrhosis). Symptoms are usually related to blood alcohol concentration (BAC) and, in cases of severe AAI, respiratory depression and arterial

hypotension can lead to death (Caputo et al. 2019). The fatal dose of alcohol is extremely variable, but the consumption of a standard drink induces a BAC of approximately 0.2 g/L, while a BAC higher than 300 mg/ dL is frequently associated to an increased risk of respiratory depression and arrest, and AAI generally occurs at a BAC higher than 500 mg/dL (Schuckit, 2006; Vonghia et al., 2008). In the majority of AWS cases, minor symptom complex (generalized hyperactivity, anxiety, tremor, retching sweating, nausea, tachycardia, systemic hypertension, mild pyrexia: peak 10–30 h) may occur after 6–8 hours alcohol interruption, sometimes associated with auditory/visual hallucinations, that may last for 5–6 days. The offset takes place in 40-50 hours (Morgan 2015). Only 5% of patients with AWS present delirium tremens (coarse tremor, agitation, fever, tachycardia, profound confusion, delusions and hallucinations), with an onset 48–72 hours following abrupt cessation or a substantial reduction in alcohol intake. Sometimes in delirium tremens, it may be present also hyperpyrexia, ketoacidosis and profound circulatory collapse if not effectively curtailed. Delirium Tremens may be fatal if not effectively treated (Morgan 2015). It is, therefore, essential that clinicians working with inpatients in ED know how to prevent, recognize, and treat AAI and AWS to minimize costly hospitalizations and avoidable death. In the event of a mild/moderate form of AAI, there is no necessity of drugs administration, but it is fundamental to monitor vital functions, keeping the patient under observation (preventing the onset of AWS) and to administer liquids in case of dehydration. Severe form of AAI may be characterized by respiratory insufficiency and coma with the consequent necessity to implement mechanical ventilation. Specific antidotes (naloxone for the use of opioids; flumazenil for the benzodiazepines-BDZs) should be administered in the case of the simultaneous use of other sedative drugs. Finally, to reduce the blood alcohol and acetaldehyde concentrations, it is possible to administer drugs (such as metadoxine) that can lead to a more rapid resolution of AAI. Health status can be achieved principally by rest and hydration. The initial phase of AWS must be promptly treated to reduce the risk of complications and to prevent convulsions and deaths, introducing BDZs (lorazepam and oxazepam, BDZs with a shorter half-life, for the elderly patients AWS

treatment) or for patients with advanced liver cirrhosis) with supplementation of non-pharmacological supports (hydration, correction of hypoglycaemia and electrolyte balance, vitamin B group supplements). Moderate forms of AWS may benefit from a treatment composed by sodium oxybate, tiapride, and clomethiazole. When BDZa are not effective in controlling specific persisting symptoms of AWS, alfa2-agonists, beta-blockers, and neuroleptics should be associated, while in the refractory forms, the association with anti-convulsant, analgesic, anesthetic drugs such as phenobarbital or propofol is recommended (Caputo et al. 2019).

Opioids Withdrawal: (D'Onofrio et al. 2015)

Dependence on prescription opioids and heroin is a major public health problem that is increasing in the United States and also internationally (Scavone, Sterling, and Van Bockstaele 2013). Opioid-dependence adverse health consequences are a frequent reason for the use of ED medical care (Cai et al. 2010; Kanny et al. 2012). Opioid abuse imposes a significant economic burden on society by increasing health care costs, unemployment rates, absenteeism, and premature mortality dependence and impacts on the drug user (Shah, 2020). Some studies highlighted that opioid abuse consequences can cost on average, 0.2% to 2.0% of a country's gross domestic product (Rehni, Jaggi, and Singh 2013). Opioids drugs are used around the world for the management of severe pain but are also commonly used as psychoactive substances, which include morphine, heroin, oxycontin, codeine, methadone, and hydromorphone hydrochloride. Opioids produce mental relaxation, pain relief, and euphoric feelings (Rehni, Jaggi, and Singh 2013), even if a chronic use may lead to the development of incapacitating form of dependence in users (Rehni, Jaggi, and Singh 2013). Treatment for the substance use disorder, comorbid medical and psychiatric conditions, or acute illnesses and trauma are the main causes of ED access for an opioid abuser (D'Onofrio et al. 2015). In a patient who is dependent on opioids, opioid withdrawal occurs when he suddenly reduces or stops taking opioids. The aetiology of opioid withdrawal is complex. Mu, delta, and kappa are the three types of opioid receptors, G protein-coupled receptors inhibiting adenyl cyclases in various tissues, that decrease cyclic

adenosine monophosphate levels. The mu receptor is crucial for reinforcing the actions of opioids. The principal site in the brain that triggers an onset of opioid withdrawal syndrome is the locus coeruleus at the base of the brain, where neurons are noradrenergic and have increased number of opioid receptors (Shah, 2020). The NAergic activity in locus coeruleus neurons, opioid receptor linked mechanism, is a prime causative site of opioid withdrawal symptoms, even if another research has also shown that grey matter and raphe Magnus nucleus are also involved in the presentation of opioid withdrawal syndrome (Rehni, Jaggi, and Singh 2013). Opioid withdrawal syndrome is a life-threatening condition resulting from opioid dependence, characterized by aversive physical and emotional symptoms. Rhinorrhea, yawning perspiration, dilated pupils, anxiety and restlessness, nausea and vomiting, diarrhoea, increased heart rate or blood pressure, as well as a host of flu-like symptoms such as chills, joint and muscle aches, and increased body temperature are characteristic signs of opiate withdrawal (Scavone, Sterling, and Van Bockstaele 2013; Wesson and Ling 2003). In opiate-dependent individuals, the experience of a pronounced and prolonged withdrawal syndrome often contributes to renewed illicit drug use and less-than-favourable treatment prognoses. It can also be caused when a patient has an opioid in his/her system and is given an opioid partial agonist like buprenorphine or antagonists like naloxone or naltrexone (Shah, 2020). Methadone and buprenorphine, two opioid agonist drugs, seem to be the most effective treatment of patients affected by opioid dependence, associated with individual and societal benefits (WHO 2009) Gowing et al., 2011). Buprenorphine is a treatment for opioid use disorder that decreases withdrawal symptoms, craving, and opioid use and that can be prescribed by appropriately trained physicians. Currently, the primary option available to the ED for opioid dependence is a referral to addiction treatment services. The introduction of buprenorphine/naloxone, a partial opioid agonist combined with an antagonist, may provide ED physicians the opportunity to initiate effective medication treatment in conjunction with a brief intervention and referral (Sullivan and Fiellin 2008).

Stimulants (Cocaine, Amphetamines)

Cocaine and amphetamine use is a major worldwide problem, with over 19 million estimated cocaine users and 33 million methamphetamine users (Richards et al. 2017). The use of cocaine has increased during the last decade, particularly in some countries, and cocaine is now the second most used illicit drug in Europe, after cannabis, with different level of use. A noticeable diversity among cocaine users is observable, in terms of use and sociodemographic characteristics, with consequent difficulty in assessing the prevalence in the use of the drug, evaluation of social impact and adoption of adequate responses. Cocaine-related health consequences are difficult to establish (Wesson and Ling 2003). The risks associated with injecting cocaine use is associated with blood-borne viruses infection (hepatitis C virus (HCV) and human immunodeficiency virus (HIV) and an elevated risk of overdose, while used in combination with opioids appears to be linked to a higher risk of opioid overdose (EMCDDA - European Monitoring Centre for Drugs and Addiction - 2007). Cocaine use has been associated with a variety of medical complications (Dinis-Oliveira et al. 2012), depending on acute or chronic use and the route of administration. The predominant pattern of cocaine use in Europe is the polydrug use, in combination with heroin, alcohol and/or cannabis, that, together with the various impurities and cutting agents, may increase the adverse health effects (EMCDDA - European Monitoring Centre for Drugs and Addiction - 2007). Cocaine benzoylmethylecgonine ($C17H21NO4$) is an alkaloid, extracted from the leaves of Erythroxylon coca (Lange and Hillis 2001), which increases the activity of monoamine neurotransmitters in the central and peripheral nervous system by blocking reuptake pumps (transporters) of dopamine, norepinephrine and serotonin (Rothman et al. 2001). The concentration of these neurotransmitters in the presynaptic cleft is enhanced and cocaine modulates preprodynorphin and the μ-, and κ-receptors of the endogenous opiate system (Kreek et al. 2005), leading to a feeling of increased energy, alertness, intense euphoria and decrease of tiredness, appetite and sleep (Rothman et al. 2001). Increased doses or a more efficient route of administration of cocaine may cause unwanted effects such as fear, irritation, panic attacks, paranoia, impaired judgement, delusions,

disturbance of sleep, weight loss and hallucinations (Rothman et al. 2001). Cocaine can be smoked, snorted or used intravenously (Lange and Hillis 2001; Rothman et al. 2001). The peak effect occurs between 1 to 90 minutes, depending on the route of administration and there are two forms of cocaine: cocaine base ('crack', 'freebase') may be smoked while cocaine salts cannot be smoked.

Acute and chronic cocaine use can cause:

- cardiovascular disorders (ischemia, acute coronary syndrome, arrhythmias, etc.);
- cerebrovascular disorders and neurological impairment (cerebrovascular accident or stroke, and status epilepticus);
- psychiatric disorders (euphoria, dysphoria, agitation, anxiety, suicidal thoughts, paranoid psychosis and depression);
- respiratory disorders, either acute (pulmonary oedema, pulmonary infarction, hemoptysis) or chronic (e.g., pulmonary hypertension);
- genitourinary and obstetric disorders, either acute (acute renal failure, mediated by rhabdomyolysis or direct toxicity, testicular infarction, placental abruption, spontaneous abortion) or chronic (premature birth, growth retardation);
- gastrointestinal complications (mesenteric ischemia or infarction);
- musculoskeletal and dermatological disorders.

Dependence is also one of the negative consequences of cocaine use, mainly in the long term (Wagner and Anthony 2002), especially for cocaine injection and crack cocaine use, associated with the highest health risks. Almost 40% of patients affected by cocaine dependence recover without treatment (Cunningham 2000).

Treatment of a cocaine intoxication is prevalently supportive, with isolated necessity of a specific intervention, such as patients with an acute coronary syndrome, in which the administration of oxygen, and sublingual nitroglycerin or verapamil are fundamental.

Both nitroglycerin and verapamil have been shown to reverse cocaine-induced hypertension, coronary arterial vasoconstriction, and tachycardia. In the treatment of an acute cocaine intoxication administration of a benzodiazepine has a prominent place, whereas β-blockers are contraindicated, while in the case of combined use of cocaine with heroin or benzodiazepine, administration of naloxone or flumazenil may be dangerous (Vroegop et al. 2009).

Both amphetamine and methamphetamine are chiral compounds with two enantiomers (mirror images) that have distinct pharmacological profiles. The levo (l) isomers of amphetamine and methamphetamine are much less active in the CNS than the dextro (d) isomers are, which is why the l-isomers may be found in pharmaceutical preparations such as Vick's inhalers. Significant absorption of methamphetamine can occur via the inhalational, oral, or intravenous (IV) routes.

Amphetamines were the second most commonly abused illicit drug worldwide according to the World Health Organization in 2007. Common complaints of ED patients with amphetamine-related visits include trauma, complications of sympathomimetic toxicity, and psychiatric issues, such as the well-documented amphetamine-induced psychosis phenomenon (Pomerleau et al. 2012). Methamphetamine, also known as meth, ice, and crystal meth, is an important drug of abuse, that can be inhaled, taken orally, or taken intravenously. Its effect lasts about eight hours, and drug testing has many false-positive and false-negative variable results. In lower doses, methamphetamine can cause an acute "fight or flight" reaction (diaphoresis, mydriasis, nausea, and tachycardia), while in higher doses may provoke psychosis and paranoia, along with hyperthermia, cardiotoxicity (including hypertension, coronary vasospasm, and aortic dissection), neurotoxicity (seizures, stroke), and acute renal disease (rhabdomyolysis, vasospasm) (Young, Weiss, Roth, and Velez 2019). Treatment is primarily with benzodiazepines.

Hallucinogens (Lysergic Acid Diethylamide or LSD, Psilocybin, Methylenedioxymethamphetamine or Ecstasy, Mescaline, Synthetic Cathinone Derivatives or Bath Salts, Phencyclidine or Angel Dust, Cannabis, Synthetic Cannabinoids Such As K2 or Spice, Bromo-Benzodifuranyl-Isopropylamine or Bromo-Dragonfly)

Hallucinogens have been used for millennia as part of ritual and religious activities. The first synthetic hallucinogen, lysergic acid diethylamide (LSD), was synthesized in 1938 by the chemist Albert Hofmann. Its hallucinogenic properties were recognized by accident in 1943 when Dr Hofmann was inadvertently exposed to LSD while working in his laboratory (Hofmann 2013). LSD was initially marketed as an anaesthetic agent and touted as an adjunct for psychoanalysis. In the 1960s, LSD emerged as a recreational drug. Its popularity peaked in the late 1960s and early 1970s and has been declining since. The drug was banned under United States federal law in 1966. There is some renewed interest in therapeutic applications for LSD, including management of treatment-resistant depression, substance use disorders, severe depression and anxiety related to terminal illness (Dos Santos et al. 2018).

Hallucinogens are used for their so-called psychedelic effects, even if hallucinogenic substances rarely produce true hallucinations, but rather primarily distort body image, sensory and time perception, mood, and thought patterns, users experience also intense and rapid mood alterations, increased intensity of any emotions, and heightened suggestibility. Hallucinogens are perceived as safe by the public but can cause dangerous physiologic effects resulting in serious health consequences. Most hallucinogens produce sympathomimetic effects, including tachycardia, hypertension, mydriasis, hyperthermia, and diaphoresis, but these are generally mild (Blaho et al. 1997). Nausea and vomiting are common and often precede the onset of hallucinogenic effects.

The proliferation of “designer drugs”—chemical analogues or derivatives of illicit drugs marketed to circumvent existing drug laws—is a growing legal problem, because manufacturers of designer drugs, stamping product with “not intended for human consumption” advisories or by identifying the products as plant food, bath salts, or potpourri, try to circumvent U.S. federal drug laws (Ng et al. 2019). Drug-induced psychosis may be difficult to distinguish from primary psychotic disorders and a patient with substance-induced psychosis is more likely to have a diagnosis of dependence on any drug, report visual hallucinations, and have a history of parental drug abuse.

Hallucinogens have been supplanted as the most common hallucinogen by the so-called new psychoactive substances, such as synthetic cannabinoids (e.g., K2, spice) and "club drugs," and naturally occurring hallucinogens, such as psilocybin and Salvia divinorum (Hoover et al. 2008; Ng et al. 2019). This is thought to be related to several factors, including the decreased supply of LSD, the rapid emergence of new psychoactive substances, and the ready availability of other hallucinogens via the internet (Malley 2019).

For a patient with hallucinogens assumption who accesses to ED because of psychomotor agitation, it is important to assess the patient’s general medical condition and to stabilize the vital signs, to identify and correct hypoxia and hypoglycemia, to obtain a core temperature, to recognize hyperthermia, to evaluate serum chemistries and to exclude rhabdomyolysis (through creatine phosphokinase analysis). It seems to be fundamental also the electrocardiogram (ECG) to identify QT interval prolongation, myocardial ischemia or arrhythmias. These patients need continuous monitoring. Hallucinogens are rapidly absorbed so gastric decontamination is not needed in most cases (Jang 2019). Adverse effects do not present until several hours after the drug was taken.

However, oral activated charcoal administration is useful for ingestions occurring within the previous two hour or longer when gastric emptying is delayed (e.g., nutmeg ingestion). Benzodiazepines are the preferred agents for the treatment of hallucinogenic-induced agitation and delirium because they possess no significant drug interactions, have no dystonic or anticholinergic adverse effects, even if reassurance and calm, or a supportive environment seem to be sufficient to soothe the agitated patient. Sometimes, to ensure the safety of the patient and the ED staff and to facilitate evaluation and treatment, pharmacologic sedation and possibly physical restraints may be necessary (Jang 2019)

Assessment

The patient evaluation should include:

History

If possible the clinician should take time to listen to the patient's history, recording baseline mental status, history of prior psychiatric illness and hospitalizations, prior violent episodes, and functional status (Caplan 2010; Gottlieb, Long, and Koyfman 2018b). Acute factors (substance use, fevers, trauma, ingestion, withdrawal, and presence of systemic disease) should be assessed (Gottlieb, Long, and Koyfman 2018a); Stowell et al., 2012)A prior history of the immunocompromised state, cancer, or neurologic disease (e.g., stroke, multiple sclerosis) can suggest a medical aetiology (New, Tucci, and Rios 2017). The examination of the patient's mental status is recommended, focusing on the patient's behaviour, appearance, affective state, thought process, presence of suicidal or homicidal ideations, attention, psychosis, awareness, judgment and insight, executive functions, and reliability (Gottlieb et al., 2018a).

Focused Physical Examination

May include close assessment of patient hemodynamics, core temperature, and cardiopulmonary and neurologic systems Medical and surgical causes for the patient's alteration, such as infection, trauma, and focal neurologic deficits should be investigated (Caplan 2010; Gottlieb, Long, and Koyfman 2018b).

Laboratory Testing and Imaging

Evaluation of point-of-care glucose level (hypoglycemia can present with a myriad of symptoms, and agitation is a well-known presentation), basic metabolic panel, toxicology screening panel (ethanol level, stimulants, hepatic function studies) and hormones studies. Head Computed Tomography (h-CT) and ECG should also be obtained if possible. Also, all women of childbearing age should have a pregnancy test performed. If patients demonstrate significant psychomotor agitation, a creatinine kinase level should be considered to assess for rhabdomyolysis (Gottlieb, Long, and Koyfman 2018a).

Scales

Scales available to assess the patient's level of agitation (Nordstrom et al., 2012) include the Behavioural Activity Rating Scale (Swift et al. 2002), Overt Agitation Severity Scale, and Overt Aggression Scale (Silver and Yudofsky 1991). Other scores primarily evaluated for sedation in critically ill patients may also be used for agitation, including the Richmond Agitation-Sedation Scale and the Sedation-Agitation Scale (Riker, Picard, and Fraser 1999). The Behavioural Activity Rating Scale is rapid and reliable for the ED, with scores > 5 associated with agitation; it does not require the patient to answer questions, which can be challenging in many patients when acutely agitated (Swift et al. 2002). Cognitive abilities can be evaluated using the Folstein Mini-Mental State Examination or the Brief Mental Status Examination (Kaufman and Zun 1995; Folstein, Folstein, and McHugh 1975).

Management

Many different management strategies exist to approach the agitated patient in the ED.

De-Escalation Techniques

Offering environmental changes, food, or other comfort measures are verbal de-escalation techniques useful before giving any sedative agents (Richmond et al. 2012). It is important to bring the patient to a quiet and safe location, in a room devoid of other patients, medical supplies, or loose objects, which may become potential weapons in the hands of the patient, and large enough to make patients feel less trapped. Additionally, to calm patients decreasing the ambient noise, dimming the lights, and adjusting the temperature are important factors (New, Tucci, and Rios 2017). Furthermore, de-escalation techniques seem to increase the likelihood that the patient will cooperate with oral dosing or facilitate the injection.

Pharmacologic Interventions

If verbal de-escalation is ineffective, medications may be required that may be given via the oral (preferable when patients are cooperative, to decrease the potential for provider injury and to increase the patient's sense of autonomy), intramuscular, or intravenous route. Different pharmacologic interventions are used in the ED setting, such as first-generation antipsychotic agents (FGA), like haloperidol, commonly utilized for the sedation of agitated patients, especially those with a known psychiatric history. Haloperidol is a typical, butyrophenone-type antipsychotic with a high affinity for D2 receptors in the brain(Li, Snyder, and Vanover 2016). It has a mean time to sedation of 25–28 min, with a mean total sedation time of 84–126 min. Second-generation (atypical) antipsychotics (SGA) are currently prescribed in place of more typical agents, due to a better side-effect profile and for a greater efficacy on dysphoria after initiation of FGAs. Most common SGAs include olanzapine, ziprasidone, aripiprazole, and quetiapine. These agents work at the level of D2 receptors, similar to FGAs, but also have an affinity for several other sites, including the 5-HT2A,

histamine, norepinephrine, and a-2 receptors. When compared with FGAs, SGAs have demonstrated similar overall efficacy. Benzodiazepines work directly at the gamma-aminobutyric acid receptor to increase sedation. Lorazepam and midazolam are the most common agents utilized in practice. Midazolam can be via the intravenous, intramuscular, intranasal, rectal, and oral routes and has a mean time to sedation of 13–18 min with a very rapid onset of action. The combination of haloperidol with benzodiazepines has been suggested to be superior to either agent alone. Ketamine is a sedative agent that works by interacting with a variety of receptors, including N-methyl D-aspartate, nitric oxide synthase, and multiple opioid receptors. It has an onset of action of 2–3 min with a duration of effect ranging from 5–30 min. The use of a ketamine-based protocol resulted in a significantly lower rate of intubations in this high-risk environment (Richardson et al. 2019; Wilson, Pepper, et al. 2012).

Restraints

They should be reserved for patients who remain a danger to themselves and others despite the above measures. Regrettably, a large proportion of agitation-related injuries has been observed during the physical act of placing the patient into restraints. Moreover, continued fighting against restraints can lead to muscle breakdown and rhabdomyolysis and risk of deaths from asphyxiation, strangulation, and chest compression due to restraint use. It is important to secure the restraints to the frame of the bed, rather than the side rails, which are mobile and to have a minimum of five people present to assist with placement. If all four extremities are to be restrained, the clinician leading the team should ensure that one arm is fixed to the gurney in the up position, whereas the other arm is in the down position, to reduce the risk of patients generating enough force to overturn the gurney. The minimal restraints that are necessary to protect the patient and others should be utilized, and patients should be closely monitored by staff and more quickly as possible restraints should be removed (Allen et al. 2005; Cleary and Prescott 2015).

AIMS

A vast body of literature has pointed to a major role of alcohol and drugs in the presentation of psychomotor agitation, especially in the ED, but no systematic review has been carried out before the current one on this topic. In recent years, there has been a dramatic rise in the incidence of alcohol and substance abuse, but only not all physicians are trained enough on the management of intoxicated patients who present agitation.

The objective of the present chapter is to report a systematic review of studies conducted in the ED on the association between alcohol or drugs abuse and psychomotor agitation. The review aimed to:

1) Identify the study designs and sampling features, as well as the measures used to determine the level of agitation and metabolic or neurological parameters alterations in alcohol or drug use.
2) Summarize the findings on prevalence estimates for different drugs used among ED patients.
3) Examine the management choices in the ED setting.

METHODS

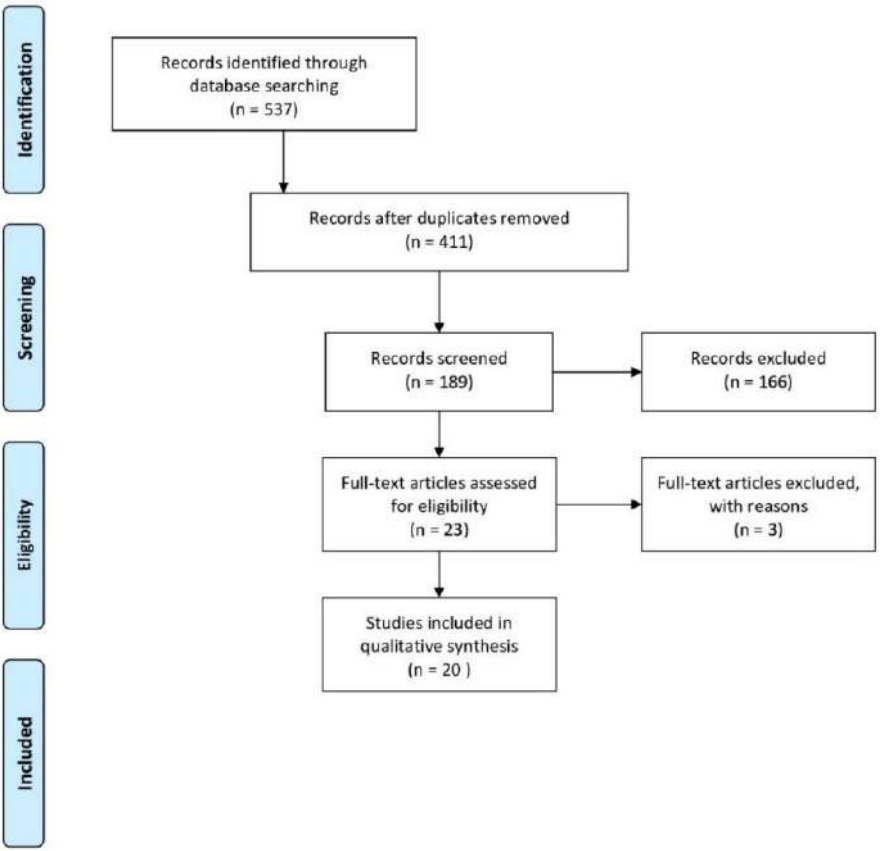
Adhering to PRISMA guidelines (Liberati et al. 2009), a literature search was conducted in MEDLINE and EMBASE on 16 December 2019, using respectively the following search string: (((((("Alcoholic Intoxication"[Mesh]) OR "Substance Abuse Detection"[Mesh]) OR "Substance-Related Disorders"[Mesh]) AND "Violence"[Mesh]) OR "Psychomotor Agitation"[Mesh]) AND "Emergency Medical Services"[Mesh] and ((alcohol OR drug) AND {psychomotor agitation})) AND ({emergency room} OR {emergency department}). The search was restricted to the English language. To be included in the review, papers had to be cross-sectional or cohort studies designed to analyze violent behaviours and/or psychomotor agitation in the context of ED associated to

use of alcohol or drugs. Two reviewers (LS and SB) independently triaged the titles and then the abstracts to exclude those that were inappropriate. A possible disagreement between reviewers was resolved by joint discussion with a third review author (EG).

After selection of the relevant studies, reviewers extracted and tabulated data using a standard form. Extracted data were tabulated (Table 1) and included: author and year of publication, country and study period, setting and study design, data source, participants' features, intoxication type, agitation and assessment methods, the test used, outcome and main results. Where necessary, text descriptions were used to highlight information that was not captured in Table 1. The need for an Ethics Committee approval was waived since we just collected and synthesized data from previous clinical trials in which informed consent had already been obtained.

RESULTS

The Scopus and PubMed literature searches identified 537 articles. After the title, abstract, and eventually full-text screening, 20 papers (Beck et al. 2015; Wilson, MacDonald, et al. 2012; MacDonald et al. 2010; Derlet and Duncan 1971; Martel et al. 2016; MacDonald et al. 2012; Wilson et al. 2013; (CDC) 2011; Yap et al. 2017; Migon et al. 2008; Knott, Taylor, and Castle 2006; Spain et al. 2008; Lung et al. 2016; First, n.d.; S. F. Li et al. 2013; Downes et al. 2009; Wilson, Chen, et al. 2012; Pepa et al. 2017; Moritz et al. 1999; Rund et al. 2006; Cole, Klein, and Martel 2019) met inclusion criteria for this review (see Figure 1 for more details). Three studies were excluded because they were not clinical trials. Narrative data extracted from the papers included in this review are reported in Table 1.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(7): e1000097. doi:10.1371/journal.pmed 1000097.

For more information, visit www.prisma-statement.org.

Figure 1. PRISMA Flow diagram ED intoxication.

Table 1. Narrative table

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Kai MacDonald, 2012	USA San Diego October 1, 2003 - December 1, 2006	2 EDs Multicenter retrospective study	Computerized ED pharmacy database; postintervention documentation	N = 146 Patients who received either IM haloperidol or olanzapine with or without concomitant medications during the study period. Male: N=95 (65%). Patient age, mean 41years (16.8 SD) *Triage complaint:* psychiatric related, N= 80 (55%)	Psychomotor agitation - Alcohol or drug intoxication (blood sample, urine sample)	4 Groups: haloperidol 5 mg intramuscular (IM) with or without a benzodiazepine and olanzapine 10 mg IM with or without a BDZ. Clinical Global Impression scale (CGI) for agitation/ psychosis: Clinical Global Impression Improvement (CGI-I) and Clinical Global Impressions	*Primary outcome:* Efficacy based on the need for additional medication intervention (AMI) in real-world agitation. *Secondary Outcome*: Severity of agitation and adverse effects	Presence of drugs and/or alcohol, N= 84 (58%). AMI was required within the 3-h period after access by 43% (13/30) when haloperidol was given alone and by 18% (13/72) when haloperidol was given with a BDZ.

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
						Severity scales (CGI-S) for Adverse Events (AEs), which balances clinical efficacy and adverse events		AMI was required by 29% (6/21) of patients receiving olanzapine alone and by 18% (2/11) of patients given olanzapine plus a BDZ. A significant percentage of patients had non-psychiatric triage complaint, drug/alcohol use, severe agitation.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Marc L. Martel, 2016	USA Minneapolis January 1, 2014 - July 1, 2014	ED (urban Level I trauma centre) Retrospective single-centre review of consecutive patients who received intravenous (IV) olanzapine in one	Electronic medical record (EMR, Epic, Verona, WI) by searching the medication administration record for "IV olanzapine"	Primary Indications for IV Olanzapine (N = 713) of which acute agitation (all etiology) N = 245 (34.4%)	Positive breath/blood alcohol test N = 118 (16,5%) mean level 0.196 mg/dL. Urine drug screens using immunoassays with confirmatory high-performance gas and liquid chromate-graphy and mass spectrometry were obtained on 23 patients.	The times of administration of these medications were recorded and then divided into four categories: given > 2 hours before IV olanzapine, given < 2 hours before IV olanzapine, given < 2 hours after IV olanzapine, or given > 2 hours after IV olanzapine. For every agitated patient, all doses of additional olanzapine (IV, IM, oral), ketamine, BDZ,	Evaluation of use and safety of IV olanzapine in the ED patient population.	N = 118 (16.5%) patients were positive for ethanol (median alcohol concentration determined by either breath analysis or serum concentration was 0.196 mg/dL), and N=7 drug screens were positive for sympathomim etics (ampheta-mine: 2,

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
						and haloperidol were documented.		cocaine: 3, both amphetamine and cocaine: 2). Thirty-four admissions (4.5%) were to intermediate or intensive care unit beds. No patients died and no cases of sudden cardiac death were noted.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Michael P. Wilson, 2011	USA San Diego January 1, 2004 - December 31, 2006	Structured retrospective chart review	Web charts, an electronic charting system that tracks all patient times, laboratory results, and clinician charting	N = 96 intoxicated agitated patients (N=71 treated with haloperidol, N=25 with olanzapine)	Evaluation of hypotension in agitated patients receiving haloperidol with or without BDZs	Measurement of vital signs and ethanol levels in patients who received haloperidol with or without BDZs compared to patients who received olanzapine with or without BDZs.	Evaluation of the appropriateness of combination of antipsychotic with BDZs for intoxicated agitated patients	Olanzapine alone or with a BDZ was not associated with more hypotension. Olanzapine plus BDZs were associated with lower oxygen saturations than haloperidol plus BDZs in Ethanol (ETOH+) but not ETOH- patients. In patients with known alcohol ingestion,

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
								haloperidol, haloperidol + BDZs, or olanzapine alone may be better choices for treatment of agitation
Kai MacDonald, 2010	USA San Diego 2003-2006	Retrospective chart review of 105 agitated ED patients	Chart review	N = 105 patients who were either drug and/or alcohol positive (N=76) or drug and/or alcohol negative (N=29).	Positive for drugs or alcohol [D/A(+)] D/A(+) patients with urine drug screen (UDS) positive for amphetamines, cocaine, alcohol or marijuana, or presence of	Patients who received either IM haloperidol or IM olanzapine, comparing prescribing patterns, level of agitation, response to treatment and side effects in patients [D/A(+)	Evaluation of the presence of drugs and alcohol in the agitated population treated with IM olanzapine or IM haloperidol	The haloperidol–BDZ combination was the most frequently prescribed treatment, although alcohol (+) status biased clinicians toward using

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
					alcohol (positive breathalyzer or blood alcohol level result). UDS results indicating BDZs or opioids were not coded as positive.	and patients D/A(−). N=46 of charts had pretreatment levels of agitation and posttreatment side effects (rated using CGI).		haloperidol alone. D/A(+) patients were rated as more agitated and had more posttreatment sedation than D/A(−) patients. In D/A(+) patients, haloperidol+ BDZ and IM olanzapine performed better than haloperidol alone.

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
John R. Richards, 1998	USA San Diego January 1995 - January 1997	ED	All adult patients who presented to the ED acutely agitated in the study period. The datasheet for each drug were sealed in a blank with no identifying marks. An equal number of lorazepam and droperidol envelopes were generated and placed in a folder in the ED.	N=202 of whom Cocaine: N=28 Methamphetamine: N=146 Ethanol: N=98	Ethanol means level pair to 160.7 +/- 84.8 (intoxication was defined as a level 80 mg/dL)	All adult patients who presented to the ED acutely agitated. Acute agitation included patients with violent, controlled or uncontrolled muscular movement. All patients were asked stand-ardized questions concerning recent use of ethanol or other illicit drugs (data sheet compared to toxicology results)	Ethanol intoxication was present in N= 98 patients. There was no difference in sedation profile between patients with different intoxications for lorazepam and droperidol	Administration of droperidol provides a more rapid and greater sedation than lorazepam in agitated patients requiring chemical, and lorazepam is more likely to require repeat dosing than droperidol.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
			After the treating physician selected patient with non-blinded randomization.					
Michael P. Wilson, 2012	USA San Diego 2004-2010	Structured retrospective chart review, 2 academic ED	Electronic charting system that tracks all patient times, laboratory results, and clinician charting.	N = 482 patients received olanzapine N= 28 patients intoxicated	For each patient, chief complaint, vital signs, route of olanzapine, and laboratory results were abstracted. Alcohol ingestion (alcohol level not zero or appearance of intoxication)	*Primary measurements* Changes in vital signs *Secondary measurements* Proportion of administration time of olanzapine orally vs intramuscularly, olanzapine with a BDZ, and the necessity of an additional agent	28 patients (10.2%) intoxicated (ETOH average level of 185 mg/dL) (N= 15 treated with oral and N= 13 IM formulation). No significant differences in alcohol levels between patients with oral or IM olanzapine. All ETOH+ patients who	Combination of olanzapine + BDZs in patients ETOH+. IM olanzapine is associated with decreased oxygen saturation (not oral), especially plus BDZ. Oral olanzapine, oral olanzapine +

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
						for agitation within 3 hours	received oral olanzapine + BDZs were male.	BDZs or haloperidol may be safer choices.
Celene YL Yap, 2017	Australia October 2014 - September 2015	A multi-centre, randomized, double-blind, controlled, clinical trial conducted in two Australian EDs		N= 361 patients, aged 18-65 years, requiring IV sedation for acute agitation. N= 92 with methamphetamine-affected patients	Methamphetamine use self-reported or reported by accompanying persons; side effects were rated by a group of clinicians (using CGI).	Patients were randomly assigned to receive either an IV bolus of midazolam 5 mg-droperidol 5 mg combined, droperidol 10 mg or olanzapine 10 mg, with two possible additional doses (midazolam 5mg, droperidol 5mg or olanzapine 5mg).	*Primary outcome* Evaluation of patients adequately sedated at ten minutes (score ≤ 2 based on a 6- point, validated Acute Arousal Scale). *Secondary outcomes*	A midazolam-droperidol combination appears to provide more rapid sedation of patients with methamphetamine-related acute agitation than droperidol or olanzapine alone.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
							Evaluation of adequate sedation time, the need for additional doses, open-label sedative medication, the ECG QTc interval and adverse events.	The most common adverse event was oxygen desaturation.
Jon B. Cole, MD, Lauren R. Klein, MD, MS, and Marc L. Martel, MD, 2017	USA October 2011 and September 2016	Urban Level I trauma centre safety-net hospital Retrospective, observational cohort study	EMR (electronic medical record) using structured query language code.	N = 40.601 patients with acute alcohol intoxication; N= 24.319 intoxicated without sedation. N= 4.495 received multiple sedative and/or concomitant	Acute agitation secondary to alcohol intoxication	Only the administration of a single antipsychotic was analyzed. Length of stay (LOS) with and without coadministration of an anticholinergic was analyzed.	Median ED LOS for patients receiving a single parenteral dose of an antipsychotic for acute agitation secondary to alcohol intoxication	Median ED LOS significantly shortest for droperidol (499 minutes, 95% confidence interval [CI] = 493–506 minutes) than haloperidol

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
				sedation with a BDZ N= 11.787 patients received a single dose of an antipsychotic (droperidol, N = 3.790; haloperidol, N = 1.449; olanzapine, N = 6.548). Median age:42 years, 76% male, 5% admitted				(524 minutes, 95% CI = 515–537 minutes) and olanzapine (533 minutes, 95% CI = 528–539 minutes).

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Knott et al., 2006	Australia Period: n.s.	Metropolitan university hospital Double-blind, randomized, clinical trial	Health Information Department of the hospital.	N = 153 patients enrolled aged 18 to 65 years with exhibited marked agitation that required chemical restraint. N = 74 patients received midazolam N = 79 patients received droperidol.	Acute agitation because of mental illness, intoxication, or both	Patients received midazolam or droperidol, 5 mg IV, every 5 minutes until sedated. The dose and time of drug administration, patient's vital signs, ECG, agitation scale and adverse events were recorded by the nurse treating the patient.	*Primary Outcome* Time to sedation (from the initial dose of the drug until a score of 2 or less on a 6-point agitation scale). *Secondary Outcome* Evaluation of the need for subsequent sedation within 60 minutes of initial sedation, the corrected QT (QTc) interval on a 12-lead ECG, and adverse event rates.	No difference in time to sedation between midazolam and droperidol. N = 11 adverse events occurred in the midazolam group and N = 10 in the droperidol group. Three patients required active airway management; all received midazolam.

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Spain et al., 2008	Australia August 2003 - December 2005.	ED of a metropolitan hospital Prospective pilot study	Protocol-informed ED consultants or registrars. Data analysis was performed by a study member.	N= 62 patients (male: N = 35, female: N = 27). Median age: 27.8 *years*.	Severe behavioural disturbance suspected to be from psychostimulant use.	Administration of midazolam with increments of 10 mg IM or IV, every 10 minutes, up to four doses and titrated to an endpoint of rousable drowsiness (when the GCS equalled 9–15 in combination with patient cooperation and compliance). Failed safety criteria were identified as the need for airway adjunct,	Effectiveness and safety of high dose midazolam protocol	The majority of patients were effectively sedated with one or two doses of midazolam: 67% with a single dose and a further 21% with a second dose. Six and a half per cent of patients were not sedated after four doses. A GCS of 8 or less was prolonged in

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
						hypotension, respiration rate <12 breaths per, GCS 8 or less and unplanned intensive care admission or significant aspiration event.		8 patients. N = 4 airway problems requiring an adjunct were present. Recent psychostimulant use was present in only 55% after a full assessment.
Pepa et al., 2017	California January 1, 2012 - December 31, 2015.	Two academic EDs Retrospective chart review	EMR, Structured Query Language (SQL) Server Management Studio.	N = 785 patients without evidence of alcohol intoxication received risperidone in the ED, and N= 52 patients with alcohol intoxication	Acute agitation, alcohol intoxication	Risperidone administration between ED patients presenting with ETOH (+) and patients without ETOH (-)	*Primary Outcome* Comparing oxygen saturation changes before and after risperidone administration between ED	Risperidone with and without alcohol intoxication and BDZ administration had no statistically significant

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
				received risperidone.			patients presenting with ETOH (+) and EtOH (-). *Secondary Outcome* To assess the effect of BDZ co-administration on vital signs	effect on vital signs (p = ns for all comparisons)
Li et al., 2013	USA Period: n.s.	Hospital ED. Retrospective observational cohort	Hospital electronic medical database with specific attention to the type of sedative drugs, frequency of administration,	N= 1300 patients (77% male). Mean age: 44 years	Alcohol intoxication, agitation	Combination of sedatives administered IV; N=155 patients received a combination of haloperidol and lorazepam;	*Primary outcome* Incidence of adverse events in patients who received combination sedatives.	There was a single adverse event, a dystonic reaction, in the combination of sedative drugs

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
			and adverse outcomes.			N= 18 patients received a combination of lorazepam and midazolam; N= 113 patients received a BDZ; N = 6 patients received haloperidol. N= 273/292 (93%) were agitated.	*Secondary Outcomes* Comparison of adverse event rates in combination sedatives and single-agent sedatives and comparison of efficacy in combination sedatives and single-agent sedatives	(adverse event rate less than 1%). Patients who received a combination of sedatives were less likely to require a second dose of sedative medication than patients who received a single-agent sedative (21% vs 44%).

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Moritz et al., 1999	France November 1997 - May 1998.	ED in a 2.650-bed University Hospital. Prospective clinical study	N.s.	N = 58 patients (N = 30 males), Mean age: 34 years (range 17±59)	Agitation, alcohol and/or drug abuse	Overt Aggression Scale (OAS), Laboratory tests including blood glucose, alcohol (performed by high-performance liquid chromatography-HPLC) and a serum drug screening (performed by fluorescence polarization immunoassay) were carried out on samples taken within 2 h following	To assess the toxicological etiologies in agitated patients and to evaluate their initial clinical diagnosis in the light of toxicological results analysis.	N= 50/58 patients were under the influence of alcohol and/or a drug. BDZs (N = 22/58), selective Serotonin Reuptake Inhibitors (N = 5/58) and opiates (N = 4/58) were the most frequently observed. For N = 39 patients the initial clinical diagnosis was alcohol

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
						admission. Further toxicological analyses were performed the day following the patient's arrival at the ED.		intoxication, although N = 1 patient was not under the influence of alcohol and N = 16 took also benzodiazepine. Serotonin syndrome in N = 2 patients.
Migon et al. 2008,	Brazil June - December 2001	Three psychiatric emergency hospitals	TREC-Rio study (a large pragmatic randomised clinical trial investigating the effects of drug treatments for agitated/aggressive patients)	N = 301 patients with agitation due to psychiatric problems N = 144 male, N= 154 female	Agitation/ substance misuse	Use of physical restraints	To examine factors associated with physical restraint in psychiatric emergency rooms.	N = 73 patients (24%) were restrained during the first 2 h of admission. At greater risk of physical restraints; younger

Table 1. (Continued)

Study (Author Year)	Country and study period	Setting and Design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
								people (OR=1.03 for each year younger), exhibiting intense (OR=2.53) or extreme agitation (OR=7.71), thought to result from substance misuse (OR=1.75) or diagnoses other than psychosis (OR=1.88), arriving in the morning (OR=1.64).

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
								Physically restrained patients did receive a greater number of drugs (median: 4.0 vs. 3.0) and a higher load of antipsychotic medications compared with people who were not restrained (median: 358.3 mg in chlorpromazine equivalents vs. 333.3 mg).

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Wilson, 2013	USA 2003, December - 2010, November	2 EDs Multicenter retrospective study	Structured chart audit performed by one of the study investigators abstracting data from the electronic medical record.	N = 49 patients received IM ziprasidone with documented clinical data. N = 23male, N = 22 female. Mean age:45 years. N = 7 patients (14%) positive to alcohol.	Psychomotor agitation Positivity to alcohol was performed with breathalyzer or blood test.	2 Groups: ziprasidone + BDZ within 30 min and ziprasidone without BDZ within 30 min. The effectiveness of medication administration was measured indirectly by the number of patients requiring additional therapy. Vital signs were calculated before and after administration of medications.	*Primary outcome* To determine the demographics of patients receiving Ziprasidone e the frequency of prescription. *Secondary Outcome* To describe the effects of Ziprasidone IM alone and with BDZ on vital signs.	N = 16/49 received ziprasidone+ BDZ with no differences in vital signs compared to only ziprasidone. The use of ziprasidone+ BDZ didn't reduce the need for additional therapy but was associated with longer lengths of stay in ED.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
							Tertiary Outcome To analyze if the combination of Ziprasidone and BDZ had other beneficial or adverse effects. *Quaternary Outcome* To find any adverse effects of combination between therapy and ethanol intoxication.	In patients who received ziprasidone, positive to alcohol, there was a significantly greater reduction in oxygen saturation at the periphery (SpO2).
Johnson, 2016	USA January 1, 2011 - June 30, 2013	Inpatient psychiatry unit Monocentric retrospective study	Electronic medical record.	N = 201 patients with psychiatric disorders.	Psychomotor agitation Drug intoxication (urine sample)	Two Groups: patients with a psychiatric disorder, positive or negative for cannabis.	Valuate any difference in length of stay, 90-day readmission rate to the same	Cannabis use occur in younger patients, males,

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
				N = 103male (51.2%). Mean age: 39 years. 21.4% positive for cannabis.	Patients positive for cannabis were compared with negative ones.	Agitation was assessed using Excited Component of the Positive and Negative Syndrome Scale (PANSS-EC)	psychiatry unit, agitation in patients with psychiatric disorders (schizophrenia, bipolar disorder, not otherwise specified) that were positive or negative for cannabis.	with bipolar disorder and active maniac symptoms. It is also associated with shorter length of stay, agitation and additional medications (prevalently oral formulations).
Downes, 2009	New South Wales 2006 January 1 - 2006, December 31	ED Retrospective study	A database was designed, and medical data were extracted and reviewed by a single investigator.	N = 122 patients that required the activation of code black for their first access for agitation in ED.	Psychomotor agitation 89% of patients had a history of alcohol/drugs abuse or psychiatric illness.	All the patients agitated and/or aggressive for which was activated Code Black (CB)	Evaluate potential strategies to curb violence in ED and describe the characteristics	Drug or alcohol intoxications were the main causes of CB activations,

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
				N = 71 male (58%) Median age: 32 years.		(specialized team in the management of these patients)	of patients with acute behavioural disturbance and their treatment in an ED with a structured team approach.	only 10% of cases were pure psychiatric disturbance. BDZ and droperidol were the medications more used (96%). Sixty-nine % of patients required 2 or more drugs dose.
Centers for Disease Control & Prevention (CDC), 2011	USA November 13, 2010 - March 31, 2011	EDs of Michigan Retrospective study	Hospitals of Michigan communicated cases of intoxication with "Bath Salts" to PCC	N=35 patients with bath salts intoxication. Male 54%, female 46%. Median age: 28 years.	Drug intoxication N=35 patients intoxicated with "bath salt."	Including all patients with reported use of "bath salts."	*Primary outcome* To formulate public health problem report of the use of "bath salts" as a drug.	One patient died for toxicity. N=17 patients were hospitalized: N=9 was admitted to

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
			(Children's Hospital of Michigan Poison Control Center). Clinical data were extracted from medical reports.	46% of patients had a history of mental illness.	Ninety-four % of patients were positive for other drugs. Clinical features: most common agitation (66%), tachycardia (63%) and hallucinations (40%).		*Secondary Outcome* To describe clinical features and toxicity of intoxication.	the intensive care unit (ICU), N=5 to a general floor, N=3 directly to a psychiatric unit. Treatment generally included a BDZ (i.g. lorazepam) to control signs of toxicity, generally low or moderate doses. If BDZ sedation was ineffective, antipsychotics were added.

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Lung, 2016	USA July 10, 2013 - August 8, 2013	ED of an urban Level I Trauma Center Prospective observational study	A biweekly pharmacy record audit identified ED patients that were given haloperidol+BDZ. Retrospective chart review for clinical information.	N= 49 ED patients with Novel psychoactive substances (NPS) intoxication, of whom N=23 had sufficient, remaining blood samples for analysis. Age: 23-96 years. N=6 male, N=7 female.	Psychomotor agitation - Drug intoxication (blood sample)	Including all patients receiving haloperidol+BDZ, excluding insufficient biological samples. Arbitrary definition of severe agitation (haloperidol for chemical restraint). Evaluation of vital signs.	To assess the utility of performing non-targeted NPS screening amongst a selected patient population in the ED setting.	N = 23 ED patients receiving haloperidol+BDZ. N=6 patients identified with seven different NPS. No patients died. Novel. Non-targeted NPS screening in a selected ED patient population is feasible and effective in identifying NPS.

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
Beck, 2016	Sweden 2012-2015	EDs or intensive care units (ICU) of Sweden Retrospective observational study	Clinical data were extracted from medical reports collected at the Swedish Poisons Information Centre. In STRIDA project were collected blood and urine samples of intoxicated patients presenting to Swedish hospitals.	N=30 (15%) stimulant 3,4-methylenedioxy pyrovalerone (MDPV) single psychoactive substance identified in the serum or urine specimens. N= 171 other psychoactive substances (N=61 comprised of both conventional drugs of abuse, N=39 other NPS, pharma-ceuticals, and ethanol)	Drug intoxication (blood sample, urine sample)	Including all patients with α MDV intoxication. The severity of poisoning is graded with Poisoning Severity Score (PSS) Laboratory results: high variability of urine and serum concentrations.	To collect prevalence, laboratory results, clinical features and poisoning severity of MDPV intoxications.	Pharmacologi cal treatment of MDPV-positive patients primarily included: benzodiazepi nes (N= 105; 56%), haloperidol (N=26; 14%), and propofol (N=16; 8%). Only seven (4%) patients required intubation. One day in hospital for N=76 (40%) patients,

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
				detected together with MDPV. N =58 (29%) cathinone-derivative alpha-pyrrolidinovalerophenone (alfa-PVP) was detected followed by methylone (N=14;7%). Age: 18–68 (mean 36, median 35) years. 71% were males.				2 days for N=79 (42%), 3 days for N=21 (11%) patients. Main clinical manifestations: agitation, tachycardia (> 100/min), and hypertension (systolic blood Pressure > 140 mmHg). Other symptoms included: hallucinations (N=31,16%), delirium

Table 1. (Continued)

Study (author year)	Country and study period	Setting and design	Data source	Sample and features	Problem (intoxication type, agitation, assessment methods)	Intervention or exposure (test used)	Outcome	Main results
								(N=29, 15%), hyperthermia (>39° C, N=18,10%), and rhabdomyolysis (N=16,8%).

DISCUSSION

Agitation in patients attending the ED is a common phenomenon, with a high risk of having patients harming themselves, service personnel, other waiting patients and/or emergency room visitors. Furthermore, overcrowding in this setting represents not only an inconvenience of elongated wait times, but it is also associated with increased transport delays, ambulance diversion, patients leaving without being seen, medication errors, with a consequent risk of death or disability (Nicks and Manthey 2012). This situation could be extremely dangerous for patients who experience psychomotor agitation correlated to alcohol and/or substance intoxication or withdrawal.

A vast body of literature has pointed to a major role of organic or psychological factors in the development of agitated behaviours. A lot of reviews have analyzed the different symptomatologic expression of agitation in these pathologies (Wilson, Pepper, et al. 2012; Gottlieb, Long, and Koyfman 2018a; Shale, Shale, and Mastin 2003; Korczak, Kirby, and Gunja 2016), but analysis of agitation with concomitant alcohol and/or substance intoxication or withdrawal remains an unmet clinical need. Aggressive or agitated behaviours in a general hospital setting are not so common; consequently, their management strategies in the ED sometimes are not optimally organized, hindering an adequate prediction and prevention of violence.

The importance of a planned and coordinated team approach is crucial in these situations, including verbal, physical and pharmacological interventions(Korczak, Kirby, and Gunja 2016; Brayley et al. 1994).

Recent alcohol or drug intake/withdrawal may cause or exacerbate agitated behaviours that require a rapid tranquillization (Table 2). To achieve the objective of rapid tranquillization, most of the studies included in this review used antipsychotics (especially droperidol, haloperidol and olanzapine)–BDZ (most commonly midazolam, diazepam and lorazepam) combination as first line of treatment of agitated patients. Some studies used for sedation a single-agent BDZ, while other single-agent antipsychotic. In

most cases, the drugs combination appears to provide more rapid sedation of patients (Table 3).

In patients with known alcohol ingestion, haloperidol, haloperidol plus BDZs, or olanzapine alone may be better choices for the treatment of agitation. Specifically, one study (S. F. Li et al. 2013) showed that patients who received a combination of sedatives were less likely to require a second dose of sedative medication than patients who received a single-agent sedative (21% vs 44%).

Regarding different intoxication/withdrawal drugs, or olanzapine alone may be better choices for treatment of agitation (Wilson, MacDonald, et al. 2012), such as a midazolam-droperidol combination appears to provide more rapid sedation of patients with methamphetamine-related acute agitation than droperidol or olanzapine alone (Yap et al. 2017).

Table 2. Type of substance

Alcohol	Kai MacDonald, 2012; Marc L. Martel, 2016; Michael P. Wilson, 2011; Kai MacDonald, 2010; John R. Richards, 1998; Michael P. Wilson, 2012; Jon B. Cole, MD, Lauren R. Klein, MD, MS, and Marc L. Martel, MD, 2017; Pepa et al., 2017; Li et al., 2013; Moritz et al., 1999
Cannabis	Johnson, 2016
Methamphetamine	Celene YL Yap, 2017
Benzodiazepines	Moritz et al., 1999
SSRI	Moritz et al., 1999
Opiates	Moritz et al., 1999
Bath salts	Benzie, 2011
NPS (new psychoactive substances)	Lung, 2016
a-PVP	Beck, 2016

The use of BDZ alone seems to be the better choice in case of hallucinogens. Severe behavioural disturbances suspected to be from psychostimulant use (bath salts intoxication) were generally treated with low or moderate doses of BDZ (e.g., lorazepam) to control signs of toxicity, and only if the sedation was ineffective, antipsychotics were added (CDC, 2011). Spain et al. (Spain et al. 2008) showed that in case of severe behavioural

disturbance suspected to be from psychostimulant use, the majority of patients were effectively sedated with one or two doses of midazolam: 67% with a single dose and a further 21% with a second dose. Six and a half per cent of patients were not sedated after four doses.

The study by Martel et al. (Martel et al. 2016) showed that in the case of acute agitation related to alcohol or drugs abuse, endovenous (IV) olanzapine was the primary indication. For every agitated patient, all doses of additional olanzapine (IV, IM, oral), ketamine, BDZ, and haloperidol were documented. In a study published in 2016, Lung et al. described that patients with severe agitation due to novel psychoactive substances (NPS) beneficiated from haloperidol-BDZ combination without severe adverse reactions (Lung et al. 2016).

Table 3. Pharmacological interventions

Antipsychotics • Haloperidol Kai MacDonald, 2012; Marc L. Martel, 2016; Michael P. Wilson, 2011; Kai MacDonald, 2010; Jon B. Cole 2017; Li et al., 2013; Lung, 2016 • Olanzapine Kai MacDonald, 2012; Marc L. Martel, 2016; Michael P. Wilson, 2011; Kai MacDonald, 2010; Michael P. Wilson, 2012; Celene YL Yap, 2017; Jon B. Cole 2017 • Droperidol John R. Richards, 1998; Celene YL Yap, 2017; Jon B. Cole 2017; Knott et al., 2006; Downes, 2009 • Risperidone Pepa et al., 2017 • Ziprasidone Wilson, 2013	Kai MacDonald, 2012; Marc L. Martel, 2016; Michael P. Wilson, 2011; Kai MacDonald, 2010; John R. Richards, 1998; Michael P. Wilson, 2012; Celene YL Yap, 2017; Jon B. Cole 2017; Knott et al., 2006; Pepa et al., 2017; Li et al., 2013; Wilson, 2013; Lung, 2016
Benzodiazepines • Midazolam Celene YL Yap, 2017; Knott et al., 2006; Spain et al., 2008; Li et al., 2013 • Lorazepam Li et al., 2013	Kai MacDonald, 2012; Marc L. Martel, 2016; Michael P. Wilson, 2011; Kai MacDonald, 2010; John R. Richards, 1998; Michael P. Wilson, 2012; Celene YL Yap, 2017; Jon B. Cole 2017; Knott et al., 2006; Spain et al., 2008; Pepa et al., 2017; Li et al., 2013; Wilson, 2013; Downes, 2009; Lung, 2016
Ketamine	Marc L. Martel, 2016

As regards side effects, it would seem that drugs combination resulted in more severe adverse reactions, as reported in three studies. In alcohol-intoxicated patients who received ziprasidone plus BDZ (Wilson et al. 2013) and intramuscular olanzapine (not oral) plus BDZs (Wilson, MacDonald, et al. 2012; (Wilson, Chen, et al. 2012) a significantly greater reduction in oxygen saturation at the periphery (SpO2) was observed. Another study (S. F. Li et al. 2013) reported a dystonic reaction among intoxicated patients treated with a combination of sedative drugs (adverse event rate of less than 1%). The Australian study conducted by Knot et al. (Knott, Taylor, and Castle 2006) showed that among patients who presented agitation correlated to alcohol or drug intoxication, three patients who received midazolam required active airway management. In fact, some studies underscored the importance of a sudden transfer to intensive care unit (ICU) beds.

Given the fragility of these patients, in many of the studies included in the current review, a close monitoring of QT interval, oxygen saturation, temperature, blood pressure, haematological parameters, was recommended to prevent significant adverse effects, including prolonged QT interval and potential for ventricular arrhythmias, hypotension and anticholinergic effects. In a previous review (Korczak, Kirby, and Gunja 2016), it was shown that droperidol and haloperidol had Food and Drugs Administration (FDA) black box warnings imposed on their use because of the risk of the prolonged QT and the consequent risk of death due to torsades de pointes. This review pointed out that adverse events were heterogeneous between studies, but principally were respiratory side-reactions, including desaturation, airway obstruction, and respiratory depression, while cardiovascular events were less common.

In the current review, none of the studies predicted the importance of de-escalation techniques, interventions which include a trigger phase, escalation phase, crisis phase, recovery phase and depression phase, as highlighted in a Cochrane review of 2017 (Du et al. 2017) on psychosis-induced aggression or agitation. This fact could be due to impracticability of de-escalation techniques in most emergency medical settings, where "resources are strained by heavy patient loads and under-staffing, and where

a sleeping patient is better than one who needs constant observation to assess the need for tranquillization."

In our review, a Brazilian study (Migon et al. 2008) showed that among patients with agitated behaviors, the use of physical restraints during the first 2 h of ED admission was higher for younger people, exhibiting intense or extreme agitation, thought to result from substance misuse or diagnoses other than psychosis, arriving in the morning. These physically restrained patients received a greater number of drugs (median: 4.0 vs. 3.0) and a higher load of antipsychotic medications compared with not restrained (median: 358.3 mg in chlorpromazine equivalents vs. 333.3 mg).

Table 4. Physical restraints

Factors associated with physical restraints	Migon et al. 2008,

In most studies, laboratory tests including blood glucose, alcohol (performed by high-performance liquid chromatography-HPLC) and a serum drug screening (performed by fluorescence polarization immunoassay) were used to assess the intoxication type (Table 5). To detect positivity to alcohol in many study breathalyzer tests was performed.

Only in one study (Yap et al. 2017), methamphetamine use was self-reported or reported by accompanying persons.

To evaluate the agitation intensity levels or the appearance of adverse events, some studies used specific scales or questionnaires (Table 6). Two studies (MacDonald et al. 2012); (Yap et al. 2017) applied the Clinical Global Impression scale (CGI) for agitation/ psychosis: Clinical Global Impression Improvement (CGI-I) and Clinical Global Impressions Severity scales (CGI-S) for Adverse Events (AEs), which balances clinical efficacy and adverse events. In another study (Johnson et al. 2016), agitation was assessed using Excited Component of the Positive and Negative Syndrome Scale (PANSS-EC) for patients with a psychiatric disorder, positive or negative for cannabis. The PANSS-EC scale consists of five behavioural components inherent in agitation: tension, excitement, hostility, uncooperativeness, and poor impulse control. Each domain is scored 1

through 7, with 1 being "absent" and 7 being "extreme." Notably, the study hospital's "adapted" scale differs slightly from the validated PANSS-EC score where we have substituted tension for hallucinatory behaviour.

Table 5. Substances detection

Alcohol or drug intoxication: blood sample, urine sample	Kai MacDonald, 2012; Michael P. Wilson, 2011; Kai MacDonald, 2010; John R. Richards, 1998; Michael P. Wilson, 2012; Pepa et al., 2017; Moritz et al., 1999; Johnson, 2016; Lung, 2016; Beck, 2016
Breath/blood alcohol test	Marc L. Martel, 2016; Wilson, 2013
Urine drug screens using immunoassays with confirmatory high-performance gas and liquid chromatography and mass spectrometry (HPLC) Ethanol dosage: high-performance liquid chromatography (HPLC)	Marc L. Martel, 2016; Moritz et al., 1999
Self report	Celene YL Yap, 2017; Michael P. Wilson, 2012; Benzie, 2011
HPLC/diode array detector and gas chromatography-mass spectrometry	Moritz et al., 1999
Poisoning Severity Score (PSS)	Beck, 2016

The Overt Aggression Scale (OAS) was used in samples of agitated patients with alcohol and/or drug abuse (Moritz et al. 1999). The Overt Aggression Scale (OAS) is a rating scale that measures aggressive behaviours (divided into 4 categories: verbal aggression, physical aggression against objects, physical aggression against self, and physical aggression against others) in adults and children.

In Knott et al. study (Knott, Taylor, and Castle 2006), a scale was developed to monitor changes in agitation levels. Comparison was made between the time to sedation using each drug and the proportion of patients sedated at 5 and 10 minutes. The time to sedation was defined as the time from the initial dose of the drug until a score of 2 or less on a 6-point agitation scale (5, highly aroused and violent; 4, highly aroused; 3, moderately aroused; 2, mildly aroused and pacing; 1, settled; 0, asleep).

Table 6. Assessment tool for psychomotor agitation

Clinical Global Impression Y Severity (CGI-S) scale for agitation/ psychosis, CGI-S for Adverse Events (AEs), and Global CGI-I	(Kai MacDonald, 2012); Celene YL Yap, 2017
Overt Aggression Scale (OAS)	Moritz et al., 1999
Excited Component of the Positive and Negative Syndrome Scale (PANSS-EC)	Johnson, 2016

All aspects of the coordination of hospital procedures, from employer support systems, the physical design of departments, training for staff to guarantee adequate aggression management seem to be necessary strategies to curb violence in the ED (Kennedy 2005), but only one of the studies included in our review (Downes et al. 2009)reported about a specific management strategy for acute behavioural alterations and psychomotor agitation in the ED setting. As specified by Downes et al., "timely recognition of potential aggressive behaviours, early verbal de-escalation strategies progressing to the use of sedative drugs and physical restraint if required" is necessary in ED setting for acute behavioural alterations and psychomotor agitation management, especially when patients present alcohol or drugs intoxication/withdrawal (Downes et al. 2009). At Emergency Department of *Calvary Mater Newcastle* public hospital in Waratah, Australia has activated the 'Code Black Team', a specific team, including security, nursing, medical and administrative staff, to manage agitation, delirium, aggression and acute behavioural disorders, in addition to medical and nursing staff. The team included security staff and hospital security assistants. In any instance in which an ED member feels insecure for the presentation of an agitated or aggressive patient, he might activate the Code Black Team via the switchboard or via a personal or fixed duress alarm. All team members have training in conflict management techniques which include verbal de-escalation, complemented by physical restraint and pharmacological sedation as deemed necessary by the clinical staff. Security staff completed the hospital incident-reporting system to report all 'Code Black Team' activations. The activation of the Code Black team was more common for those cases of psychomotor agitation presenting in patients with a history of alcohol/drugs abuse rather than in patients with a psychiatric

illness (90% vs 10%). In the intoxicated patients, BDZ and droperidol were the medications more used (96%).

Even if it was not included in our review, particularly interesting seems to be the recent work by Vieta et al. (Vieta et al. 2017), in which a protocol was reported to guide the appropriate selection and use of pharmacological agents (inhaled/oral/IM), seclusion, and physical restraint for psychiatric patients suspected of or presenting psychomotor agitation (motor restlessness and mental tension requiring prompt recognition, appropriate assessment and management). The protocol, born from the cooperation of Spanish psychiatrists, psychologists and nurses, experts in psychomotor agitation, was based on recently issued international consensus guidelines on the standard of care for psychiatric patients with psychomotor agitation.

CONCLUSION

Alcohol or drugs intoxication/withdrawal is an intercurrent, potentially transitory condition, that can be concomitant with psychomotor agitation presentation in EDs. Even if administration of drugs is not necessary in case of a mild/moderate forms of alcohol or drugs intoxication/withdrawal, in any case, the patient must be set under observation and vital functions must be monitored to prevent some serious side events, such as respiratory insufficiency, heart attack, and coma. Sometimes specific antidotes should be administered (naloxone for the use of opioids; flumazenil for the BDZs). In the ED setting, only if the assessment is approached systematically and if communication is effective inner staff members, an adult patient presenting alcohol or substance use disorder can be managed and discharged correctly. In the evaluation process, essential steps seem to be screening for substance use and psychiatric disorders, enhancing motivation, and making an appropriate referral. An appropriate and timely treatment of agitation in alcohol or drug intoxication/withdrawal is vitally important for reducing the risk of complications and minimizing financial costs of hospitalization.

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Chapter 2

IMPACT OF ORAL HEALTH RELATED QUALITY OF LIFE IN SCHOOL CHILDREN

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ABSTRACT

The oral health-related quality of life (OHRQoL) has been defined as a "multidimensional construct that includes a subjective evaluation of individual's oral health, functional well-being, emotional well-being, expectations and satisfaction with care, and sense of self" [1]. In this chapter, the common oral health-related neglected aspects in school children like- dental caries, pain, dental trauma, developmental anomalies

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and malocclusion will be discussed including the overall impact on their daily life on the basis of academic performance, physical performance, socioemotional development. In order to address and counteract these circumstances at the primitive stage, the contemporary preventive oral health care measures and dental interventions will also be discussed. This will enlighten the parents, school teachers, nurses, coaches and oral health professionals about the basic oral health needs of school children for the overall improvement of their quality of life.

1. INTRODUCTION

Happy, healthy, active, and well-nourished children are more prone to be regular in school, more dedicated, and eager to learn and therefore; academically more achieving than the children with the compromised health condition. Oral health-related quality of life (OHRQoL) is considered as an integral part of general health and well-being. Children with poor oral health and general health are 2.3 times more likely to report poor school performance as children with poor oral health alone are 12 times more prone to restricted activities [2, 3]. In the USA it was estimated that approximately 51 million school hours were lost in a year due to oral health-related issues [4]. Among the children receiving emergency dental treatment - 19% reported having difficulty in playing, 32% with school attendance, 50% with sleeping, and 86% with eating [5]. The World Health Organization (WHO) stressed the importance of oral health not only for its physical, psychological impacts on the population but also its effect on their growth pattern, look, functional abilities like- speaking, chewing, smiling, sense of taste, ability to socialize and their overall feelings of social well-being [6]. Good oral health encourages children to acquire better physical activities, academic performance, and social skills. On the contrary poor oral health often leads to intolerable pain, functional disabilities, lower self-esteem, depression, fear of awkwardness, and the impairment of daily activities. According to WHO the major oral health concerns are- dental caries, gum diseases, malpositioning of teeth due to early loss of deciduous teeth (i.e., milk teeth), and traumatic injuries [7]. Dental erosion, developmental defects, oral cancers, soft tissue infections are also important. The occurrence of dental

caries (i.e., tooth decay) and gum diseases are so common that affected over 80% of the school children in some population and the cost of treating dental caries alone is enough for putting pressure on a country's total health care budget [3]. Early tooth loss has a deleterious effect on a child's nutritional intake which consequently impacts negatively on his/her growth and development. Although many of these oral diseases are preventable and can be cured at an early onset; there is a considerable lack of knowledge among the children, their parents and teachers. Schools are an effective establishment for providing oral health education to the children. Furthermore, schools can also play a vital role providing oral health knowledge and information to the parents and the local community. Therefore, it is important that the teachers and school staffs are enlightenend with adequate oral health knowledge and skills. Therefore, this chapter is aimed at explaining the common oral diseases and the basic oral health needs of the school-children to attain and maintain optimal oral health for overall contribution to their general health and well-being.

2. Oral Health Impact on the Daily Activities of School Children

The Child Oral Impacts on Daily Performance (Child-OIDP) is one of the most widely accepted and applied instruments to assess the OHRQoL in school children. It is the modified version of the OIDP index and since its development in 2014; it has been applied successfully throughout the world to assess the OHRQoL of school children in a different population [8]. The index measures OHRQoL on basis of eight daily performances (Table 1). It also allows measurement of OHRQoL at wider dimensions such as- oral health impairments, functional limitations, and disability [9]. In the United Kindom, 40.4% of schoolchildren reported having at least one oral impact influencing their daily activities of which difficulty in eating was most prevalent (23.2%) [10]. In Italy and France, this frequency raised to 66.8% and 73.2% sequentially with the most prevalency of difficulty in eating [11,

12]. In Malaysia, 3 out every 4 school children were identified with oral impacts affecting up to a maximum of 3 daily performance [9]. Inadequate eating leads to a nutritional imbalance which impacts negatively on the physical and academic performance of school children. Oral health conditions like- teeth sensitivity, toothache, malpositioned teeth (i.e., malocclusion), bleeding gums, erupting teeth, loss of primary teeth, oral ulcers were commonly related to the impacts [11-13].

Dental caries is mostly associated with toothache and sensitivity. Inflammation of the pulp due to dental caries is the most common human infective disease affecting 60-90% of school children worldwide [14]. Other possible causes of dental pain- traumatic injury, infection, the eruption of permanent teeth, and exfoliation of deciduous teeth [15]. In a study, it was stated that the children poor oral health are 3 times more likely to miss school than their counterpart due to toothache [16]. These school absences were associated with poor school performance. School absenteeism for regular dental care was not related to poor academic performance. At the Ontario province of Canada, schools with a higher percentage of children requiring emergency dental treatment had significantly higher proportions of children scoring below provincial averages in all six school performance catagories [17]. Due to dental pain children also suffer from emotional instability and restricted social engagement (e.g., playing time) [18]. Malocclusion affects perceived attractiveness by others and social acceptance [19]. Traumatic dental injuries, particularly of the incisor and canine teeth, deprives children of the social skills like- smiling, enjoying companies of other people as they are anxious about the others' perceptions of them [20].

Table 1. Child-OIDP Index performance factors

• Eating and enjoying food
• Spaking and pronouncing clearly
• Cleaning teeth
• Smiling, laughing and showing teeth without embarrassment (smiling)
• Sleeping
• Maintaining a useful emotional state without being irritable
• Carrying out major work or social roles (school work)
• Enjoying social interference with people

3. Major Oral Diseases in School Children

3.1. Dental Caries

Dental caries is the most common disease in school children as being over 5 times common than asthma. According to a child dental health survey (2013) in England- 31% of 5 years old children were diagnosed with dental caries in their primary teeth and 46% of 15 years old had caries in their permanent teeth [21]. In the United States, untreated dental caries was found in 15% of the children and adolescents whereas, among the minority children and those below the federal poverty threshold the percentage raised subsequentially to 20% and 25% [22].

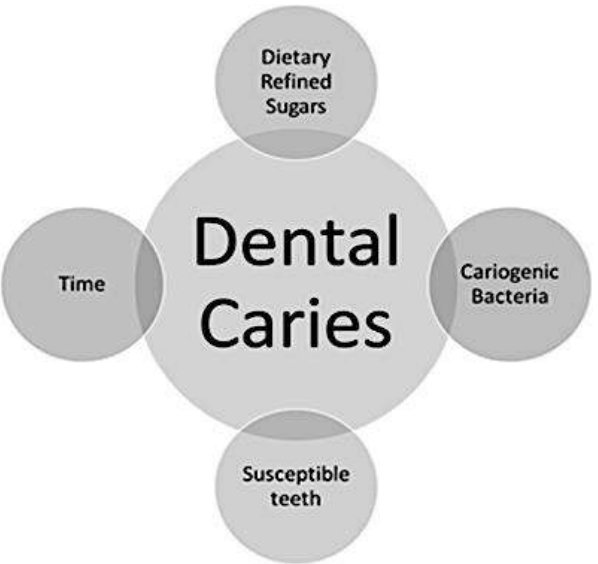

Figure 1. Four important elements responsible for dental caries.

The disease is characterized initially by the demineralization (i.e., decay) of teeth by the acids, produced by the cariogenic bacterias through the metabolism of dietary refined sugars [23]. At the primitive stage, the disease can be identified as a localized white or brown spot on the dental hard tissues (i.e., enamel and dentine). If left untreated and continuously

exposed to the overwhelming risk factors (Table 2), the spot transforms into a cavity which eventually deepens towards the pulp (i.e., soft connective tissue providing both blood and nerve supply to the teeth) and induces toothache. Consequently, acute inflammation of the pulp (i.e., acute pulpitis) and later of the periodontal tissues (i.e., apical periodontitis) evolves which are the most common causes of toothache. From the apical region of the teeth, the infection can further spread to jaw and beyond on the account of persistent negligence.

Table 2. Oral health conditions requiring emergency dental treatment in children

• Traumatic dental injuries
• Large visible cavities
• Acutely swollen gum
• Infection
• Dental pain

3.1.1. Causative Factors of Dental Caries

Although dental caries is a multifactorial disease (Table 1), it is mainly caused by the consumption of refined sugar (e.g., sucrose). The other factors can only act together in the presence of refined sugar and modify its effect. In the human diet, the principal sources of carbohydrates are- starches, sucrose, and some lactose, with less glucose, fructose, or maltose. Sucrose is mainly extracted from the sugar beets or sugar cane. Puddings, cakes, milkshakes, fruit juices, cookies, pancakes, caramels, dark chocolates, commercial cereals are highly enriched with sucrose. Ingestion of sucrose lowers the pH of the adherent plaque to a level of decaying the enamel (i.e., the outer surface of the teeth) (Figure 2) before slowly returning to the resting level. It also encourages the colonization of the *streptococcus mutans* which is considered as a main cariogenic bacteria. Rather than at a single dose, if sucrose-containing drinks are consumed repeatedly at short intervals it helps to feed the bacteria more frequently with the newer substrate (i.e., sucrose) and thereby, the acidity of the plaque persists at a destructive level. A similar mechanism also applies fo the sticky foods (e.g., caramel,

chocolates, cakes) that clings onto the teeth surface, slowly dissolves, supplying the substrate.

Table 3. Required factors predisposing dental caries

Dental plaque	White, soft, and tenaciously adherent deposit on the teeth surface containing a dense concentration of bacteria and their bi-products with the ability to concentrate and retaining acid.
Acid-producing Bacteria	Viridans streptococci (e.g., *Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarious, Streptococcus mitior, Streptococcus sanguis*).
Stagnation areas for plaque	Pits and fissures on the occlusal surface of molars, lingual pits of maxillary incisors approximal surfaces of incisors and canines.
Vulnerable teeth	Molar-incisor hypomineralisation newly erupted teeth until post-eruptive maturation.
Time	Plaque (i.e., prerequisite for dental caries) appears when the teeth surfaces are left unbrushed for 12-24 hours and the process of caries starts.

Table 4. Risk factors related to dental caries in school children

• Poor oral hygiene
• Lower socioeconomic status
• Lack of maternal education
• Higher frequency of dietary sugar intake
• Enamel hypoplasia (developmental defect characterized by the lack of enamel)
• Reduced salivary flow
• Lack of supplemental fluoride intake

3.1.2. Consequence of Dental Caries

At the earlier stage, as the dental caries are asymptomatic, they may remain undetected. But as the disease progresses from the enamel into the dentine (Figure 2); it carries sensation to the pulp tissue evolving mild to moderate toothache. Therefore, early diagnosis is important as the progression of the disease can be intercepted at this stage. Reminarealisation is also possible in a favorable environment with necessary fluoride supplements provided. Untreated dental caries leads to an inflammatory condition of the pulp termed ‘irreversible pulpitis.’ The pain of irreversible pulpitis is so severe than it often keeps the children awake at night and only

partly relieved by analgesics. This requires emergency dental interventional treatment. On further avoidance of the treatment, the pulp tissue becomes necrosed and the affected tooth appears to be asymptomatic for a while but eventually, a dental abscess may develop with swelling and further pain [24]. The infection worsens, spreads into the jaw bone and adjacent area of the neck, and develops into cellulitis (i.e., soft tissue infection). The condition can be fatal as the soft tissue swelling may obstruct the airway leading to oxygen deprivation and suffocation commonly known as Ludwig's angina [25].

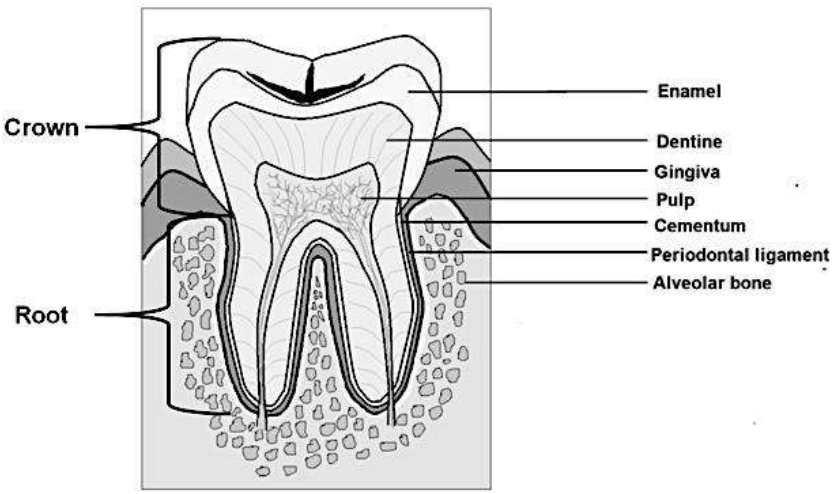

Figure 2. Basic anatomy of a tooth and surrounding structures.

3.1.3. Prevention of Dental Caries

3.1.3.1. Role of Diet

Consumption of fermentable carbohydrates should be reduced. The WHO recommends the total intake of free sugars to be less than 10% of the total energy intake. Fluorides from the drinking water or other sources can prevent caries. The fluoride content of the drinking water should be 1 ppm (parts per million) or more to be effective against dental caries. Fluorides can also be supplemented by toothpaste, mouth rinses, milk, salt, etc. Sucrose can be replaced by the sugar substitutes like caloric (e.g., xylitol,

sorbitol, maltitol, mannitol) and non-caloric sweeteners (e.g., aspartame, cyclamate, acesulfame-K, saccharin). They are little or non-fermentable to oral microorganisms, resulting in restricted acid production.

3.1.3.2. Role of Parents

Parents should assist their young children in practicing oral hygiene measures. Supervision should be initiated at least once in a day preferably just before bedtime. While toothbrushing various techniques can be adopted like- standing behind the child or having the child's head placed in the parent's lap [26]. Parents should be aware of the association between the frequent consumption of fermentable carbohydrates and dental caries and act promptly to restrict the consumption of highly cariogenic foods and promote healthier snacking. Parents must seek professional dental screening for their children every 6 months interval.

3.1.3.3. Role of School

School-based dental education programs can effectively reduce the incidence of dental caries in the community by raising awareness. These may include- necessary oral hygiene instruction, demonstrating oral hygiene practices, the importance of conserving the primary dentition (i.e., milk teeth), and permanent teeth, promoting oral health-friendly dietary habits. Schools can arrange preventive oral health measures requiring professional assistance like- community dental screening, topical fluoride application, use of fluoride tablets and rinses, sealing caries susceptive teeth surface, etc.

3.1.3.4. Role of Oral Health Care Providers

Oral health professionals can apply high concentration (12,300-22,600 mg/kg) fluoride gel, foam, or varnish to the teeth once or twice yearly [26]. Thorough dental check-ups are necessary to detect susceptible teeth surfaces and early carious lesions. Teeth surfaces that are prone to dental caries should be sealed using sealants (e.g., occlusal sealants). Early caries can be intercepted and restored by using fluoride-releasing cement. Regular oral health measures (e.g., toothbrushing technique, flossing, etc.) should be rechecked with both the patient and the parents.

3.2. Diseases of the Periodontium

The periodontium is composed of the gingiva (i.e., gum), periodontal ligament, cementum of the tooth root and the alveolar bone (Table 5 & Figure 2). The prevalence of gingival inflammation (i.e., gingivitis) among the children ranges between 2.2% to 100% [27]. In the United States, 82% of adolescents have gingivitis and signs of gum bleeding [28]. In most countries around the world, children above 7 years of age were diagnosed with gingivitis [27]. Among the periodontal diseases, plaque-associated chronic gingivitis and periodontitis are most prevalent in children and adolescents up to the age of 20 years [28].

Table 5. Epidemiological factors influencing the prevalence and severity of the periodontal diseases

Socio-economic status	Lower socioeconomic groups have a higher prevalence of the periodontal disease.
Age	The prevalence and severity of periodontal disease reaches a maximum with the onset of puberty and decreases in late puberty
Sex	Boys have a higher prevalence and severity of gingival inflammation than the girls
Geographical differences	People in rural areas are more susceptible than in urban areas.
Ethnicity	The diverse ethnic groups have different prevalence and severity of periodontal diseases.

3.2.1. Chronic Gingivitis

Chronic gingivitis is characterized by the inflammation of the gum without any detectable loss of underlying alveolar bone or attached connective tissue fibers (i.e., periodontal ligament). At the initial clinical investigation, chronic gingivitis exhibits redness and swelling of the marginal gingiva and bleeding on probing. Eventually, the edematous gingival tissue becomes fibrotic, interdental papilla becomes enlarged and bulbous, and the probing depths gradually increase. Accumulation of dental plaque is mainly responsible for this disease. Many predisposing factors encourage plaque accumulation (Table 6). Accumulated plaque calcifies to

form calculus. Calculus can be seen most commonly at the sites of lower incisors and upper molars. Calculus cannot be removed by the patient and it provides a rough retentive surface for more plaque accumulation [29]. Steroid hormone, various drugs such as – cyclosporine, phenytoin, calcium channel blockers, etc. are also found to be responsible for the gingival overgrowth and subsequent inflammation [30]. Steroid hormone-induced gingivitis is associated with increased levels of sex hormones (i.e., estrogen and progesterone) during puberty.

Plaque induced chronic gingivitis in children should be managed by the mechanical plaque removal (i.e., tooth brushing, flossing) and high levels of oral hygiene maintenance. Therefore, dentists and dental hygienists should educate both the parents and their children regarding the importance of oral hygiene. For the children who are physically challenged or undergoing orthodontic treatment- electric toothbrushes and antibacterial mouth rinses may be useful to aid in plaque removal. The twice-daily dose of chlorhexidine rinses may be prescribed when normal brushing and flossing fails to achieve plaque control.

Table 6. Predisposing factors for dental plaque accumulation

Malocclusion
Dental caries
Orthodontic appliance
Inadequate restoration
Mouth breathing

Table 7. Glossary on the anatomy of the periodontium

Gingiva	Part of keratinized oral mucosa covering the alveolar bone and the cervical (i.e., lower portion of the crown) portion of the teeth.
Periodontal ligament	Connective tissue fibers attaching the tooth roots to the alveolar bone
Cementum	Calcified tissues covering the tooth root.
Alveolar bone	Wall of bone housing the teeth.

Scaling (i.e., removal of plaque and calculus) and polishing of the teeth should be scheduled at regular intervals to eliminate the bacterial deposits.

Severe cases gingival overgrowth (e.g., steroid hormone and drug-induced gingivitis) requires surgical intervention (i.e., gingivoplasty or gingivectomy) to recontour the gingiva for hygienic and esthetic reasons.

3.2.2. Periodontitis

Gingivitis does not always lead to periodontitis, but periodontitis is the descendant of gingivitis [30]. In periodontitis, there is a loss of periodontal tissue attachments and the destruction of supporting alveolar bone surrounding the teeth. Like gingivitis, the accumulation of dental plaque is mainly responsible for periodontitis. Two types of periodontitis are common in school children- pre-pubertal periodontitis, and, juvenile periodontitis. Both pre-pubertal and juvenile periodontitis are aggressive periodontal diseases and may be localized or generalized. The onset of pre-pubertal periodontitis occurs between 2.5 years to 10 years of age and it's prevalence ranges between 0.84 to 26.9% [31]. Juvenile periodontitis occurs in children and teenagers; characterized by rapid bone loss around one or more permanent teeth in otherwise healthy individuals. In a study, it was estimated that- among the American schoolchildren aging between 13-17 years- 10% African-American, 5.5% Hispanic, and 1.3% white adolescents had juvenile periodontitis [32].

3.3.3. Local and Systemic Factors Predispose to Periodontal Disease in Children and Adolescents

A) Local factors:
   a) Oral infectious disease: herpetic gingivostomatitis, necrotizing ulcerative gingivitis, etc.
   b) Oral pathosis: cysts, fibromas, granulomas, hemangiomas, oral Kaposi sarcoma.
   c) Iatrogenic: faulty dental restorations, mishaps during endodontic treatment.
   d) Habits: rubbing or picking the gum using a fingernail.
   e) Trauma: Injury to the periodontal ligament caused by trauma.

f) Mouth breathing: dryness of the gingiva results in reduced blood flow and hence, reduced microbial resistance.

Table 8. Diagnostic criteria and management of prepubertal and juvenile periodontitis

	Localized prepubertal periodontitis	**Generalized prepubertal periodontitis**	**Localized juvenile periodontitis**	**Generalized juvenile periodontitis**
Onset	Around 4 years or older	During tooth eruption	Around puberty	Late teen years
Affected teeth	Either few or many teeth	All primary teeth, permanent teeth may or may not be affected	Permanent incisors and/or first molars	Generalized involvement of permanent teeth
Gingiva	Minor inflammation if any	Fiery red gingiva with acute inflammation around all teeth, gingival proliferation, cleft formation, and recession.	Gingiva appears healthy but may bleed on gentle probing.	Severe gingival inflammation with multiple and recurrent abscess formation.
Dental plaque	Minimal	Greater plaque accumulation	Minimal, not corresponding to the amount of bone destruction	Heavy accumulation of plaque
Alveolar bone destruction	Faster than adult periodontitis but slower than generalized prepubertal periodontitis	Rapid	Bilateral angular bone loss around molars or incisors	Severe generalized bone loss
Familial distribution	Indicated	Indicated	Indicated	Indicated
Systemic diseases	Either neutrophils or monocytes may be affected but not both. Otitis media and upper respiratory infection in some cases.	Both neutrophils and monocytes are affected, marked increase in peripheral white blood cell count, otitis media, and upper respiratory infection in most cases.	Not identified	Not identified
Management	Antibiotic therapy, oral prophylaxis, and mechanical removal of plaque and calculus (i.e., scaling).		Antibiotic therapy (tetracycline or amoxicillin and metronidazole combination), mechanical removal of subgingival plaque and calculus (i.e., scaling and root planing)	

B) Systemic factors:
   a) Hormonal changes during puberty and menstruation.
   b) Systemic diseases: diabetes mellitus, Down syndrome, chronic renal failure, AIDS, otitis media, upper respiratory infection, impaired neutrophil or monocyte chemotaxis, leukocyte adhesion deficiency, leukemia, secondary hyperparathyroidism, Papillon-Lèfevre syndrome, Chèdiak-Higashi syndrome, acrodynia, scleroderma.
   c) Nutrition deficiencies: riboflavin, folic acid, vitamin C, and niacin.
   d) Systemic drugs: Azathione, phenytoin, nifedipine, cyclosporine.
   e) Heredity.

3.3 Oral Lesions

The most commonly found oral lesions in school children are- primary herpetic gingivostomatitis, recurrent herpes simplex infection, recurrent aphthous stomatitis, diffuse intraoral candidiasis, angular cheilitis, geographic tongue, and traumatic lesions. Children suffering from systemic diseases like- asthma, diabetes, immunodeficiencies, blood diseases, encephalopathies, organ transplantation, genetic syndrome, heart, and renal diseases are more prone to have oral lesions [33]. In the US, the most prevalent oral lesion in school children was lip or cheek bite (1.89%) followed by recurrent aphthous stomatitis (1.64%), recurrent herpes labialis (1.42%) and geographic tongue (1.05%) [34]. The most commonly affected sites were- lips (30.7%), the upper surface of the tongue (14.7%), and the buccal mucosa (13.6%). Moreover, lesions were more prevalent in boys (11.76%) than girls (8.67%).

3.3.1. Recurrent Aphthous Stomatitis

This ulcerative oral lesion is commonly referred to as ‘crank sores’ by the patient. The size ranges between 0.5-3 cm in diameter. The ulcer is

shallow, well-defined round or ovoid with a grey-yellowish central area surrounded by a reddish halo. They are commonly seen at the labial and buccal oral mucosa or sides of the tongue. Common predisposing factors include- trauma, stress, menstruation, nutritional deficiencies, food allergies, hormonal diseases. Found as a common manifestation in children diagnosed with Behcet's disease and HIV-infection [35]. Small lesions usually heal within 7-10 days without any scar; larger lesions take weeks to heal with definite scarring. Treatment options are topical anesthetics, antimicrobial mouth rinses (e.g., 0.12% chlorhexidine), topical corticosteroids, and topical tetracyclines.

3.3.2. Traumatic Lesions

Traumatic lesions occur due to injuries caused by habits (e.g., biting lips and cheek), faulty dental restorations, orthodontic appliance, biting after the admission of local anesthetic, chemical burns (e.g., hypochlorite), burns from foods, etc. The ulcers are usually tender with yellowish-grey floor and red margins. On elimination of the cause, the ulcers usually heal within a few days. Acidic

3.3.3. Geographic Tongue

It is also termed as "Erythema Migrans.' The appearance of the lesion is like a world map on the upper surface of the tongue and hence, known as Geographic tongue. It is a benign inflammatory condition. An occasional burning sensation may occur. The cause is unknown. There is no effective treatment but spicy and acidic foods can be avoided to relieve the symptom. The condition may resolve by itself after a long time or recurrent for decades.

3.3.4. Primary Herpetic Gingivostomatitis

It is mainly caused by the type 1 herpes simplex virus. The virus usually transmits through close contacts (e.g., saliva). Children under 6 years are mostly affected. Vesicles appear favorably at the gum, hard palate, and upper surface of tongue approximately 1 week after the transmission. The vesicles are 1-3 mm in diameter, dome-shaped, tensed, and filled with clear fluid. Within a day or two, the vesicles rupture and leave shallow ulcers.

Fever and swollen cervical lymph nodes associates. Oral lesions usually resolve within 7-10 days. Infected children fail to eat or drink, sleep poorly, and become grumpy. So, supportive treatments like- bed rest, soft bland diet, drinking through a straw, and paracetamols are important. Chlorhexidine mouthwash is useful for pain relief and oral hygiene maintenance. Treatment with an antiviral drug (e.g., aciclovir) is highly effective.

3.3.5. Recurrent Herpes Labialis

It is a secondary infection known as 'cold sores' caused by the reactivation of the latent herpes simplex virus in an already immune person. Reactivation may occur in 30% of the population. Triggering factors may include- common cold, other febrile conditions, ultraviolet light exposure, menstruation, stress, trauma, immunosuppression. The lesion appears as vesicles at the mucocutaneous junction (i.e., vermillion border) of the lips extending onto the adjacent skin. The vesicles enlarge, combine, weep exudate, and eventually ruptures after 2-3 days. The new vesicles appear and this cycle continues for approximately 12 days. Treatment includes- application of antiviral drugs such as 5% aciclovir cream on the affected sites before vesicles appear. The prescribed application of penciclovir every two hours is more effective. Aggressive treatment with antivirals is effective in patients who suffer from frequent, multiple, and large cold sores.

3.4. Traumatic Dental Injuries (TDIs)

3.4.1. Epidemiology

Traumatic dental injuries (TDIs) are the most common (92%) manifestation in individuals seeking consultation or treatment due to trauma to the oral region. Dental injuries mostly involve tooth fracture, avulsion, or subluxation (Figure 3 and 4). It was estimated that- one out of every two children aged between 8 to 12 years sustains a dental injury [36]. Around the world, approximately 33% of preschool, and 25% of schoolchildren suffer from traumatic dental injuries involving both permanent and deciduous teeth [28]. In a prospective study, it was estimated that 30% of

children had injuries to the deciduous teeth and 22% to the permanent teeth [37]. The peak incidence of dental injuries is at 2-4 years and 8-10 years of age. Boys are generally more prone to injuries than girls due to the more active participation in contact games and sports [38]. Untreated dental injuries lead to pain, loss of function (e.g., chewing, biting), esthetic problems that impact the children physically, emotionally, and socially. They become targets for harassment and teasing by other schoolchildren and often have nicknames [39]. Because of the injury, the children are restricted to home activities and lose school hours due to absenteeism [40]. In summary, children with untreated traumatic dental injuries (TDI) are 20 times more likely to have a lower quality of life than children without any TDIs [41].

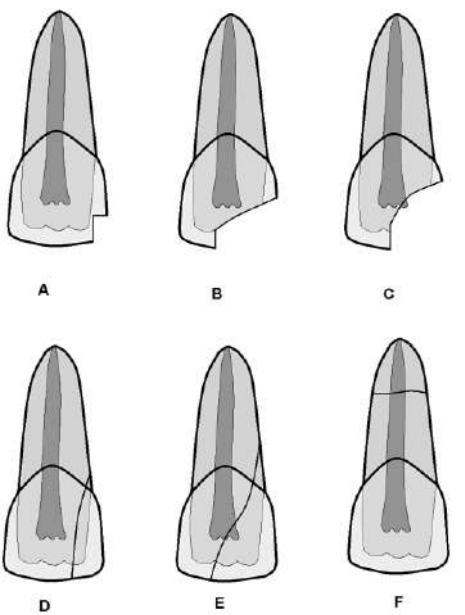

Figure 3. Types of tooth fractures- A) Involving enamel only, B) Involving both enamel and dentine, C) Complicated crown fracture involving pulp, D) Crown-root fracture, E) Complicated crown-root fracture, F) Root fracture.

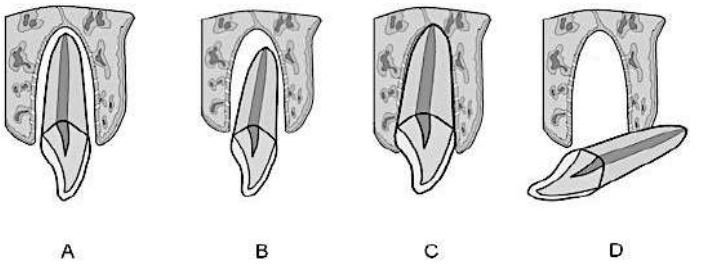

Figure 4. Types of tooth fractures- A) No displacement, B) Extrusive luxation, C) Intrusive luxation, D) Avulsion.

3.4.2. Types of Traumatic Dental Injuries

A crown fracture involving only enamel is the most common type of traumatic dental injury in permanent dentition. A fracture involving both enamel and dentine also follows. Other types of dental injuries are less common. The majority of these dental injuries involve upper front teeth, particularly the central incisors which is also the common site of injury in the primary dentition. However, in primary dentition injuries are mostly confined to the supporting structures (i.e., luxation and exarticulation).

3.4.3. Predisposing Factors

a) Protrusion of upper incisors. (e.g., class II malocclusion)
b) Inadequate lip closure.
c) Environmental factors: unsafe playgrounds, sports facilities, streets, schools, and houses; deprivation and overcrowding.
d) Behavioral factors: hyperactive and risk-taking children, children with a peer relationship problem.

3.4.4. Etiology

A) Unintentional injuries:
   a) Falls and collisions.
   b) Physical leisure activities (i.e., sports)
   c) Traffic accidents.
   d) Inappropriate use of teeth. (e.g., cutting or holding objects, opening screw top bottles)

e) Biting hard items. (e.g., piercing jewels)
f) Physical illness or limitations. (e.g., epilepsy, cerebral palsy, anemia, dizziness, impaired hearing).

B) Intentional injuries:
a) Physical abuse. (e.g., child abuse, battered child syndrome).
b) Iatrogenic procedures. (e.g., prolong intubation, injuries during the dental procedure under anesthesia).

3.4.4. Prevention of TDIs

Table 9. Suggested review chart for athletic trainers or those who are responsible for children and teenagers in play [42]

Term	Type of injury	Immediate treatment	Refer to a dentist
Crown fracture (Uncomplicated)	Part of tooth broken, no bleeding	None	Within 48 hours if the patient has difficulty due to cold sensitivity.
Crown fracture (Complicated)	Part of tooth broken and bleeding from the fracture site.	None, do not place any medication on the broken pulp, stop bleeding with sterile cotton gauge.	As soon as possible
Root fracture	The tooth might appear normal in position but bleeding from the gum around the tooth. The crown of the tooth might be pushed back or loose.	None	As soon as possible
Concussion and subluxation	Tooth still in its normal place and firm or slightly loose.	None	Within 48 hours for evaluation only.
Luxation	Tooth very loose and/or has moved from its normal position	Only move the tooth to a normal place if easy to move.	As soon as possible, especially if it is not possible to reposition the tooth to its normal place.
Avulsion	A tooth completely moved out of the mouth.	Replace the tooth in its socket, if not possible then store it in milk or saline	Immediately, it is important for prognosis.

By taking adequate measures around 7-25% dental injuries can be prevented [42]. Oral and dental injuries can be best prevented by educating-how to avoid them and how to manage them initially at the site of occurrence. Education is equally important for both children, teenagers, and those who are usually around them (e.g., parents, school officials, youth leaders, coaches). A simple instruction sheet (Table 9) is provided to ensure emergency care at the site of injury. Special attention should be given to those who are at risk (e.g., class II malocclusion, protruded upper incisors). Preventive orthodontic treatment can be initiated in these individuals before the age of 11 years to reduce the risk of trauma. Young children who are prone to repeated injuries require special counseling and attention.

3.4.5. Devices to Prevent TDIs

Using faceguard and/or mouthguard during sports can prevent or at least significantly reduce the impact of dental injuries. Faceguards are usually a .pre-fabricated cage' made of metal or composite attached to a helmet. Mouthguards are custom-made, protective devices worn inside the mouth to protect the teeth, gums, and lips from injury. Before it was made mandatory to wear faceguard and mouthguard, facial and oral injuries in US high school football constituted up to 50% of all reported football injuries [42]. Mouthguards are also indicated during oral endoscopy or oro-tracheal intubation to prevent unwanted dental injuries.

Table 10. Protective measures in different contact sports

Sport	**Protection**
American football	Helmets and facemasks significantly reduce the chance of oral injury.
Basketball	Mouthguards significantly reduce the risk of oral injuries.
Boxing	Mouthguards can reduce the chance of dental injuries.
Field and ice hockey	Face masks and/or mouthguards significantly reduce oral and facial injuries.
Rugby	Mouthguards can protect against oral injuries.
Soccer	Goalkeepers and forwards may require mouthguard protection.

Traffic accidents can cause severe injury to the head, oral, and facial region. Bicycle and motorcycle riders are exposed to serious head injury

combined with dental and soft tissue wounds during accidents. Bicycle helmets are usually designed to prevent head injuries and offer no protection against the lower facial and dental injuries. Helmets with chin-bars are more likely to protect the lower half of the face. Wearing safety belts reduce the frequency, severity, and morality of the facial injuries caused by car accidents.

3.4.6. First-Aid Management of TDIs

The general people recognize traumatic dental injuries as a common accident in children while there is a considerable lack of general knowledge regarding appropriate first-aid management in these situations. Emergency first-aid treatment is necessary to limit the damage and improve the treatment outcome. The importance of publicizing and promoting preventive measures and first-aid practices to limit the emotional effect, preserving teeth, and reducing short and long-term expenses cannot be underestimated.

Table 11. Emergency first-aid management of TDIs

Primary teeth	**Permanent teeth**	
	Crown fracture	Avulsed tooth
Injuries may often involve adjacent soft tissue. Therefore, wash the wounds with plenty of running water. Stop bleeding by compressing the injured area with gauze or cotton for 5 minutes. Seek emergency treatment from a pediatric dentist.	Find the tooth fragment and keep it wet by keeping it in saline, saliva or milk. Seek dental treatment immediately.	Calm the patient Find the tooth and pick it up by the crown If the tooth dirty, wash it briefly (10 seconds) under running water and reposition it. If not possible, store the tooth in a glass of milk. The tooth can also be transported in the mouth, keeping it between molars and inside of the cheek. Avoid storage in water. Seek emergency dental treatment immediately.

3.5. Dental Anomalies

To ensure proper oral health, care should be started from home. Tooth development starts from the very beginning of life (i.e., the fifth week of

intrauterine life) [43]. Parents should be aware of the timing of tooth eruption, shedding, numbers of the tooth in per dentition period, and also should rule out any deformities by the regular dental visit. However, three stages are observed throughout human life.

1. Primary/Deciduous dentition.
2. Mixed dentition.
3. Permanent/Adult dentition.

In the first and second stages, a child requires support from the parents and school health care providers to ensure a healthy oral cavity.

3.5.1. Primary/Deciduous Dentition

Primary teeth are often known as "milk teeth," or "temporary teeth" or in the medical term "deciduous teeth.' These are the first set of teeth seen in the mouth that fall off or shed and eventually are replaced by permanent teeth. Primary teeth usually start to appear in the oral cavity at about the age of 6 months and are completed by 28 ± 4 months [45]. Table 12 shows the approximate eruption age of primary teeth [46]. But it may vary between one-two months. There are 20 teeth in total during the primary dentition. Among the four quadrants (upper right, upper left, lower right, and lower left) of the oral cavity; five primary teeth are present in each quadrant- two incisors, one canine, and two molars.

Table 12. Eruption time of primary teeth

Tooth	Upper	Lower
Central Incisor	10 months	8 months
Lateral Incisor	11 months	13 months
Canine	19 months	20 months
1st Molar	16 months	16 months
2nd Molar	29 months	27 months

To avoid tooth decay care should be taken from a very early age. Parental counseling plays a vital role in this regard. Up to three-four years of age children usually stay at home or go to the daycare center or pre-school. Following points are to be maintained during this age period [46]:

1. Caries can be prevented by cleaning the milk teeth with a clean, soft cloth dipped in warm saline. Each time the child takes milk, teeth should be cleaned.
2. Finger brush can be used during bathing to clean the teeth.
3. Breastfeeding is encouraged as it aids in jaw (temporomandibular joint) development.
4. Bottle feeding should never be introduced during sleeping; it encourages nursing bottle caries.
5. No sugar should be added to the bottle milk.
6. Drinking from glass should be encouraged after 1 year of age.
7. Bottle feeding habits should be withdrawn completely by the age of two years.
8. By this age child should be habituated to brushing two times daily; after breakfast and after dinner.
9. By the age of three years, the complete dentition of primary teeth (total 20 teeth) should be present in the mouth.
10. The child should get three full meals a day.
11. Parents should check if the child is growing any habit like thumb suckling, lip suckling, lip biting, nail-biting, tongue thrusting, oral breathing, etc.

3.5.2. Shedding

At about five-six years of age, children start experiencing loss of baby teeth. This process is known as “shedding” or “exfoliation.” This is a result of the pressure created by the permanent teeth trying to emerge in the oral cavity. This pressure and following sequential events resorb the root of milk teeth and they eventually fall out. It is a physiological process. During this period children need to be assured that their teeth will ultimately be replaced with big, strong teeth. There is nothing to worry about. Some myths or

popular fantasy are present in society, like the "Tooth Fairy" concept to comfort children.

During this period children usually experience the first dental visit which is very important. The first impression of the dentist makes a strong impact on the child for the rest of life. It is better for them that they get a friendly approach, reassurance, and experience less/no pain.

3.5.3. Permanent/Adult Dentition

These are the teeth the child will carry for the rest of their life. They emerge in the oral cavity after shedding of the milk teeth. They are total 32 in number in both jaw; eight teeth in each quadrant. Central incisor-1, lateral incisor-1, canine-1, premolar-2, molar-3. However, for shedding and eruption, each tooth has different timing (Table 13) [47].

Now, the timetable may vary from person to person more or less 6 months to one year. However, if a tooth does not erupt timely parents should consult with the dentist to rule out any anomalies.

Table 13. Eruption timing of permanent teeth

Tooth	Upper	Lower
Central Incisor	7-8 years	6-7 years
Lateral Incisor	8-9 years	7-8 years
Canine	11-12 years	9-10 years
1st Premolar	10-11 years	10-12 years
2nd Premolar	10-12 years	11-12 years
1st Molar	5-6 years	5-6 years
2nd Molar	12-13 years	12-13 years
3rd Molar	17-25 years	17-25 years

3.5.4. Mixed Dentition

This is the period of life when the temporary teeth start shedding and the permanent teeth are erupting. This period is most important for proper occlusion, beautiful smile, and future appearance.

Why shedding, eruption, and number, size, and form of tooth is so important! Each dental arch of human is capable of containing 16 teeth, which is normal. But sometimes there are crowding, spacing, extra tooth,

absent tooth, and structural abnormalities that can also be found. Any of these abnormalities if noticed earlier can be treated early. This will free the child from the bullying leading to low self-esteem, depression, and anxiety, and in some cases, physical symptoms also develop [48]. The causative factors behind these anomalies can be a genetic inheritance, metabolic, or environmental in origin [49]. In the following sections, each of the anomalies is described in brief.

3.5.5. Abnormality with the Eruption

The eruption of baby teeth starts approximately at about six months of age is completed by two years of age. Variations are found in different races, different families, and also due to some environmental factors such as nutritional causes. However, the parent should be concerned if it passes more than a year from the eruption time table and should consult with the dentist. Another thing parents should always check that if the permanent tooth is erupting behind the primary tooth. It is a common scenario in the case of incisors and is one of the major causes of a crooked tooth.

'Natal teeth' are the teeth present at birth or erupts shortly after. It may be the temporary lower incisors or maybe an extra tooth (supernumerary). Removal of the tooth or smoothening of the sharp edges is done to aid feeding. In most cases, these teeth are lost and thereby extracted or removed.

The delayed eruption may be caused by localized and generalized causes. The tooth may be congenitally absent, attached to the underlying bone (ankylosed), does not have enough space to erupt, another tooth is obstructing its path of eruption. The tooth may be in an abnormal position or there may be a cyst/tumor preventing it from the eruption. The most common cause is that the primary teeth are still present in the oral cavity so the permanent tooth could not erupt. All of these are localized causes.

As a result of delayed eruption malocclusion develops, which will be discussed later.

3.5.6. Abnormalities with the Number

3.5.6.1. Missing Teeth (Hypodontia)

Hypodontia is the term used when there is a tooth missing due to developmental failure. Sometimes, there is a complete lack of teeth in one or both arches (Anodontia), which is relatively rare. If six or more teeth are missing, the condition is known as oligodontia [50].

The most commonly missing tooth is the third molar which does not create a major concern if first and second permanent molars are present. The maxillary lateral incisor is the second most commonly missing tooth. As this tooth is seen during the smile it has a strong impact on a child's appearance. The treatment options to replace missing lateral incisors are canine substitution, tooth-supported restoration, and single-tooth implantation. Treatment preference solely depends on the child's age, type of malocclusion, the space available, and the condition of adjacent teeth [45]. It is strongly advised to consult with a combination of specialists (orthodontist, restorative dentist, prosthodontist, and pediatric dentist) for treatment planning [52].

3.5.6.2. Additional Teeth (Hyperdontia)

It is a common phenomenon that children occasionally may have an extra tooth, which is known as 'supernumerary tooth.' It is usually conical in shape or malformed; present at the incisor or molar region [50]. Clinically termed as 'mesiodens' when found in between the two upper central incisors which are about 80% cases. Apart from esthetic concern supernumerary teeth are also responsible for causing some local disorders; such as deciduous tooth retention, delaying the eruption of an adult tooth, an ectopic eruption that is when the tooth is erupted in somewhere else rather than its original position, tooth displacements, cysts, and some other difficulties which require surgical or orthodontic treatment [53]. All these disorders are caused because of the supernumerary teeth occupying space in the dental arch obstructing other teeth which originally owned that space. It also forms a stagnation area increasing the susceptibility to caries, gingivitis, and periodontitis (inflammation of the gum and surrounding tissue).

3.5.7. Abnormalities with the Size

3.5.7.1. Large Teeth (Macrodontia)

Tooth larger than the normal is termed as 'Macrodont' or 'Megadont.' The upper central incisor is most commonly affected followed by lower second premolar. If generalized macrodontia is found the child should be investigated for systemic diseases. As this condition affects facial aesthesis, treatment should be started soon after eruption. Treatment should be planned by a combination of specialists (pediatric dentist, orthodontist, and restorative dentist) [52].

3.5.7.2. Small Teeth (Microdontia)

Tooth size smaller than normal is termed as 'Microdontia.' This is commonly seen in upper lateral incisors and usually affects girls. The microdont may be conical in shape which is termed as 'peg-shaped lateral.' Usually, those children suffering from hypodontia also have microdontia [52]. Treatment options are acceptance or restorative modification or placement of a crown.

3.5.8. Abnormalities with the Form

3.5.8.1. Fusion/Gemination

Rarely, a 'doubled tooth' is seen in the upper anterior segment, usually in the case of central incisor. This occurs either due to 'fusion' of two tooth germs or a single tooth germ is divided to form two teeth which is known as 'geminated tooth.' However, whichever cause lies behind it affects the child's esthetics and prone to caries involvement. This type of case demands a multidisciplinary (endodontic-surgical-periodontal-restorative) treatment approach to protect the child's oral health and obviously to restore esthetics [54].

3.5.8.2. Accessory Cusps

Accessory cusps are often found in posterior teeth, particularly in molars. It raises concern when it is associated with the incisors, the condition

termed as ‘talon cusp.’ This is an additional cusp or cusp like structure at the ‘cingulum’ (the bulging portion on the palatal side of the upper incisors). If the talon cusp is small there is nothing to worry about. But a large talon cusp is responsible for creating occlusal interference, difficulties during speech and mastication by irritating the tongue, prone to caries and periodontal diseases, creating abnormal pressure to the opposing tooth, and damaging that tooth also [50]. This tooth needs to be treated to restore the esthetics as well as for proper occlusion [52].

3.5.8.3. Invaginated Tooth

In this case, there will be depression or groove in the crown, sometimes extending very deep near to the root. It forms a stagnation area and the tooth becomes susceptible to caries. Moreover, when near to pulp or gets infected it demands endodontic treatment. This invaginated tooth is also termed as ‘dens invaginatus’ or ‘dens in dente.’ However, soon after eruption resin sealant placement is advised to prevent bacterial accumulation [52].

3.5.8.4. Evaginated Tooth

This is termed as ‘dens evaginatus’ and most commonly premolars are associated. There will be a small tubercle or elevation on the occlusal surface. If it is worn away or fractured during occlusion/ mastication may lead to pulpal involvement. Selective and repeated grinding of the tubercle or pulpotomy is the treatment of choice [52].

3.5.8.5. Taurodontism

This is a condition where the tooth contains a large body and very short roots. Commonly seen in molars in permanent dentition. Endodontic treatment of ‘taurodonts’ are extremely challenging and complex. It is usually associated with other developmental anomalies.

3.5.9. Abnormalities with the Structures

To understand this topic properly the tooth structures are needed to be described in short. The tooth is a combination of hard and soft structures (tissue). Three hard tissues are present enamel- the outermost layer covering

the crown, cementum- the outermost layer covering the root, and dentine-the hard tissue forming the body of the tooth. The soft tissue is the pulp that is responsible for the blood supply, nerve supply, and nutrition of the tooth. There are some supporting structures of the tooth, such as periodontal ligament- suspending the tooth in the socket, alveolar bone-the the underlying bony tissue forming the socket, and oral mucosa-the the soft tissue covering the alveolar bone.

3.5.9.1. Defects of Enamel

Enamel is the outermost layer of a tooth and is the hardest structure of the human body. The defect of enamel is the result of genetic or environmental factors or a combination of both. If the family history of enamel defect is present, the child is likely to develop the same defect. He/she should be observed and consult with the dentist as soon as any defect in the enamel is noticed. The defects in enamel are mainly thin enamel, pitted or grooved enamel, discolored enamel, and frosty or opaque enamel [50]. These defects are found in both temporary and permanent dentitions.

'Amelogenenesis imperfecta' is the clinical term used when enamel defect is present in all teeth of both dentitions. In this case, the enamel may appear as thin, pitted, or grooved, poorly mineralized, or hypocalcified. This is a serious condition as the child often is a victim of bullying by schoolmates and others. Sometimes the child is teased by the family also. They often get mocked by hearing that they have 'rotten teeth,' 'dirty teeth,' 'vampire teeth' etc. This not only affects the child psychologically but physically also [48]. Discriminatory behavior lowers the child's self-esteem. A common phenomenon is usually noticed as a result of bullying, the child hesitates or does not want to talk or communicate with others at all. He/she simply isolates him/herself from friends, the group works, or any social gatherings; often leading to depression, anxiety, and also deteriorates the learning skill resulting in poor performance [56]. The psychological symptoms are quite alarming and leave a serious impact on future life including vandalism, drug abuse, and aggressive behavior [57].

But these conditions are treatable. In the very early stage temporary treatment is given and after the eruption of permanent dentition is completed

these teeth get restorative treatment with or without endodontic management. If restoration cannot be done prosthodontic treatment or a single tooth implant is also possible.

3.5.9.2. Defects of Dentine

In 'dentinogenesis imperfecta' the teeth are usually discolored (grayish/brownish), opalescent and fragile. The translucency of the teeth is the characteristic feature. The cause may be genetic or may be associated with 'osteogenesis imperfecta' [58]. If not treated immediately, the enamel tends to chip away exposing the dentine. The soft, defective dentine ultimately wears away and the tooth height is reduced to the gum level. To prevent this treatment should be started as early as possible [59]. Treatment aims to maintain the height of the tooth and after the eruption is completed permanent treatment is given.

'Dentinal dysplasia' is another condition where the roots are very short or absent and the pulp chamber is obliterated. These teeth are known as 'rootless teeth' and tend to lose early in life.

These defects also raise an esthetic concern and it is the main reason for seeking treatment. However, treatment should be started immediately to protect the child from developing psycho-social problems.

3.5.9.3. Defects of Cementum

Cementum defects are comparatively rare and often associated with hypophosphatasia leading to early loss of tooth [52].

3.5.10. Systemic Diseases Affecting Teeth

- Rickets: In severe cases, it causes hypo calcification of teeth.
- Hypothyroidism: Hypoplastic, ridged enamel and short rooted teeth are associated.
- Hypophosphatasia: It interrupts bone maturation and cementum formation which leads to early loss of teeth.
- Congenital syphilis: If the fetus survives, it is born with some characteristic dental features. Hutchinson's incisor, Moon's molar,

and Mulberry molars are found in congenital syphilis. Hutchinson's incisors are the small, barrel-shaped incisors with tapering towards the tip; Moon's molars are the dome-shaped first molars, and Mulberry molars are molars with small nodules on the rough pitted occlusal surface instead of cusps.

- Congenital porphyria: The deciduous teeth become red or purple in cases with congenital porphyria [50].
- Neonatal Jaundice: Another condition is observed in the deciduous dentition of children who had severe neonatal jaundice. The teeth become yellow or have greenish color bands.
- Fetal alcohol syndrome: Another reason for developmental defects is maternal alcoholism. It delays dental development and also shows some enamel defects including mottled and/or translucent enamel.
- Rhesus incompatibility: Rhesus incompatibility is found when a mother with a negative blood group carries a baby having a positive blood group. As a result of severe neonatal jaundice green or grayish-yellow discoloration is noticed in the developing teeth.

3.5.11. Drug Interaction

- Tetracycline: Tetracycline is an antibiotic responsible for permanent discoloration of both primary and adult dentitions. It shows blue/gray discoloration of teeth. This drug must be avoided during pregnancy and childhood [52]. Tetracycline pigmentation occurs only if it is taken during the developmental period of teeth, whether it is baby teeth or adult teeth.
- Chemotherapy: Children who get cytotoxic chemotherapy as a treatment of malignancy ultimately result in some side effects on the teeth. The teeth may have a short root. Hypoplastic defects on the enamel may also be found.
- Fluorosis: Deciduous tooth may have defected enamel identified by mottled surface due to excessive fluoride content in the drinking water or excessive dose of topical fluoride or overuse of fluoride toothpaste. This condition is termed as 'fluorosis' and also found in

permanent teeth. In extensive cases, the symptoms may vary from opaque spots to discolored and pitted enamel with very thin hypoplastic structure [52].

3.5.12. Clefts of Lips and/or Palates

Some children are born with clefts on the lip or in the palates or combination of both. The risk of developing cleft lip or palate is higher in children whose one or both of the parents have such defects. A series of difficulties are faced by the child and the family and the management is multidisciplinary. It includes surgical, orthodontic, prosthodontic, and cosmetic procedures along with speech therapy and psychological counseling. Treatment should be started immediately and continued until the late teen ages [50]. Followings are prime factors of treatment planning-

- Psychological support for the family and later for the patient.
- Aid in feeding as soon as possible as the clefts of the palates prevent suckling. 'Feeding plate' or 'specially designed bottles' make it easier for the infant to swallow.
- Multiple surgical interventions are performed at different ages, starting from three months of age.
- The child should be carefully monitored for any hearing loss between the age of one to five years. Cleft patients tend to grow ear infections repeatedly for which ear grommets are required.
- Speech and language therapy is essential and should be started between the age of three and eight years.
- As children with clefts have poor oral hygiene they are at an increased risk of developing gingivitis and caries. Other dental anomalies are also common in these children. Therefore, close monitoring for any dental defect and sub sequential management is necessary.

Children having clefts leaves a strong impact on the quality of life. Girls have a significantly lower quality of life due to more social and aesthetic concerns. Their appearance and speech are mostly concerned. To introduce

public policies that support these children, aid in social acceptance, and improve their quality of life should be emphasized [60].

3.5.13. Ankyloglossia

A condition when the tip of the tongue is attached to the floor of the mouth. Also known as 'tongue-tie.' It interferes with feeding and speech. Surgical management is recommended.

3.6. Malocclusion

Malocclusion is fairly a disease, it is rather considered as a condition with a set of dental deviations [61]. Irregular teeth, crooked teeth, spacing, protruded teeth so many complaints are noticed when parents come to the dental clinic. These types of problems are referred to as orthodontics and are treated by orthodontists.

Parents need to be more familiar with the problems so that they can decide whether to seek help from the specialists or this is normal for the child. A regular dental visit also pays a great contribution is answering this concern.

Followings are some of the common orthodontic problems encountered by the child:

3.6.1. Crowding

When the teeth in the arch are crowded. They may be rotated, displaced, or malaligned. Commonly termed as 'crooked tooth.' It may be single tooth crowding or multiple teeth crowding.

3.6.2. Spacing

When there is a gap or spacing between teeth. It is normal to have space in temporary dentition, but a matter of concern in case of permanent dentition.

3.6.3. Overjet

The upper incisors lie in front of the lower incisors. In normal occlusion the distance between the tip of the upper incisor and the body of the lower incisor in 2-3mm. this distance is known as overjet. More or less to the normal overjet is a matter of concern.

3.6.4. Reverse Overjet:

When the upper incisors are behind to the lower incisors, the normal condition is reversed it is known as reverse overjet. Treatment is required.

3.6.5. Overbite

It is the length of the body of the lower incisors covered by the upper incisors. It is about 2-3 mm in normal occlusion.

3.6.6. Crossbite

In normal occlusion, the upper teeth are in front of the lower teeth. If any lower tooth stays in front of the upper tooth/teeth, it is known as a crossbite. It may be a single tooth crossbite or multiple tooth crossbite.

3.6.7. Openbite

Normally the anterior teeth of both jaw occlude and there is no gap in between them. But in case of an open bite, there remains a gap between the upper and lower teeth even after occlusion. It is most commonly noticed in the anterior segment.

3.6.8. Median Diastema

When there is the spacing between the two upper central incisors, it is known as midline diastema. There are various reasons for it. Treatment options are also multiple.

3.6.9. Class I Malocclusion

In Class I occlusion the upper first molar occludes with the lower first molar. In this case, the buccal cusp of the upper first molar rests on the groove (buccal) of the lower first molar. The overjet and overbite are normal.

3.6.10. Class II Malocclusion

In Class II malocclusion the upper first molar is in a forward position in relation to the lower first molar. The overjet is increased and the overbite may be reduced (deep bite) or increased (open bite).

3.6.11. Class III Malocclusion

In Class III malocclusion the lower first molar is in a forward position in relation to the upper first molar. The overjet is reduced or reversed and overbite is also reduced.

3.6.12. Ugly-Duckling Stage

At the age of eight to twelve-year children often develop a space between the two central incisors. This period is referred to as the 'ugly duckling stage' [62]. The central incisors flare and they are proclined than their temporary predecessors. It is normal and this occurs to accommodate the larger and wider permanent incisors [63]. Space closes gradually as the lateral incisors erupt and closed completely after the eruption of permanent canines [64].

Oral health-related quality of life (OHRQoL) describes the impact of oral health or disease or any oral health-related condition on the child's daily life, social life, physical and mental well-being, moreover in all aspects of life [61]. Malocclusion has been reported to be related to a poor OHRQoL [65]. The vital parameters of OHRQoL are physical, social, and psychological conditions of life and interestingly, these are the main reasons for seeking orthodontic treatment in the first place [66]. However, the treatment does not readily change the quality of life. An improvement of OHRQoL was noticed after the removal of the appliance used for treatment [65]. Oral health has more impacts on the daily life of children who were under treatment or did not receive any treatment than the children who had already completed their orthodontic treatment [67].

Followings are the impacts of malocclusion on OHRQoL:

3.6.13. Physical Impact

Pain commonly affects the QoL, but malocclusion is not responsible for pain directly. Rather it has a passive action in creating pain. Malocclusion is responsible for temporomandibular joint disorders which leads to pain. Deep bite, open bite, traumatic bite- all these conditions predispose to mucosal inflammation (gingivitis, palatal inflammation) causing pain. Abnormal occlusion also leads to periodontitis causing pain.

Malocclusion also increases the risk of trauma. Children often fall and damage the upper central incisors. This happens mostly on children with prominent (proclined) upper incisors [66].

Malocclusion often impairs the speech. Children are unable to pronounce some sibilants.

3.6.14. Psychological Impact

Facial appearance, dental appearance, smile – all are affected by a malocclusion. Children who are dissatisfied with their appearance often feel shameful and inferior to others. They have low self-esteem and choose to live isolated [66].

3.6.15. Impact on Social Life

Children having malocclusion experiences bullying, name-calling, teasing not only from other children but sometimes from family members also. This has a serious impact on their socializing skill. The victims often isolate themselves from social gatherings, suffer from anxiety and depression, and also develops poor learning skill [66].

CONCLUSION

OHRQoL is primarily measured by physical, psychological, and social conditions. Oral health in school children is typically affected by dental caries, periodontal diseases, oral ulcers, trauma, dental anomalies, and malocclusion. If a child has any of these conditions it surely will have an impact on his/her oral health followed by subsequent consequences on

OHRQoL. School children are at that stage of life when they are not able to make decisions for themselves or they are not aware of their well-being. Parents, babysitters, or other caregivers need to take the responsibility of monitoring their oral health care from a very early age. School-based oral health promotions are highly appreciated to improve children's OHRQoL. In case of a shortage of trained dental personnel, teachers or teaching assistants can be given a short training on oral health care guidelines for school children. Dental treatments are often neglected or avoided because of the high expense. Government policies and collaborations with other international organizations may result in an improvement in oral health care and thus raising the OHRQoL.

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Chapter 3

THE EFFECT OF HEMOLYSIS ON HEMATOLOGY LABORATORY TEST

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ABSTRACT

Hemolysis in blood samples is a problem that is commonly found in laboratories. This has a potential effect on the qualiy of blood tests, completion time or TAT (turn around time), and creates discomfort for patients due to repeated sampling. Hemolysis can occur in vitro or in vivo. In vitro hemolysis can occur due to lysis of red blood cells during the sample collection and handling of blood samples. In vitro hemolysis is a result of pre-analytical causes associated with sample collection, jarring transportation methods, extreme temperature, sample handling, delayed processing, and prolonged storage. In vivo hemolysis occurs if the rate of erythrocyte damage increases, thereby reducing the life span of erythrocytes. Hemolysis results in a decrease in the number of RBCs and

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HCT values due to lysis. Hematological instruments usually lyse the sample before measuring the HGB, the number of PLT, the number of WBC, and the number of WBC differential cells, so these values are usually not affected by hemolysis. In samples with hemolysis, the ESR values will decrease. In the ESR test with the Westergreen method, it is often found that hemolysis samples are difficult to assess because of unclear boundaries. The mechanism for shortening APTT in hemolysis samples has not been confirmed. This is thought to be caused by the release of phospholipids from erythrocytes and intracellular substances from leukocytes and platelets which can activate Cascade coagulation. Other literature states that activating the freezing cascade will cause PPT shortening and decreased fibrinogen levels, whereas APTT can extend or shorten the levels depending on whether there is activation or loss of fibrinogen. Hemolysis samples that are immediately examined may experience coagulation activation, so the APTT results are shortened.

Keywords: hemolysis, *in vitro, in vivo*

1. INTRODUCTION

Hemolysis in blood samples is a problem that is commonly found in laboratories. This has a potential effect on the quality of blood tests, completion time or TAT (turn around time), and creates discomfort for patients due to repeated sampling. Hemolysis is the most common reason for specimen rejection, accounting for approximately 25% needing to be repeated. Attention to pre-analytic variables including careful blood sampling (phlebotomy) and proper handling of samples can minimize hemolysis. Having a documented system for detecting and following up on hemolysis samples is important to ensure test quality [1].

Hemolysis samples are often seen in laboratory practice with an average prevalence of around 3% of the total samples routinely sent to clinical laboratories. One of the most cited and fundamental studies was conducted by the College of American Pathology in 10,709,701 samples from 453 participating laboratories. The results showed that as many as 37,208 specimens (0.35%) were rejected before testing because the samples underwent hemolysis [2].

Inadequate analysis of blood samples can cause errors in diagnostic and monitoring evaluations, resulting in unreliable results that negatively impact analytical quality and patient safety. Simultaneously, hemolysis samples often require the collection of new samples, delaying patient care. Therefore, it is important for clinical laboratories to conduct studies to evaluate hemolysis disorders [3].

2. HEMOLYSIS

The definition of hemolysis is the release of hemoglobin and other intracellular components as a result of damage to red blood cells. Hemolysis appears when the amount of free hemoglobin is greater than 0.3g/L [1].

Hemolysis can occur in vitro or in vivo. In vitro hemolysis can occur due to lysis of red blood cells during the sample collection and handling of blood samples. In vivo hemolysis occurs if the rate of erythrocyte damage increases, thereby reducing the life span of erythrocytes. Erythrocytes can be hemolyzed (intravascular hemolysis) mainly in blood vessels and the heart and it is clinically recognized as hemoglobinemia, hemoglobinuria, and decreased serum haptoglobin concentrations [4].

Table 1. Classification of Hemolysis samples [2]

Classification	**Free hemoglobin level in serum or plasma**	**Appearance of sample**
Non Hemolysis	≤ 0.05 g/L	Yellowish
Little hemolysis	≥ 0.05 to 0.3 g/L	Yellowish to Pinkish
Mild hemolysis	≥ 0.3 to 0.6 g/L	Pinkish
Hemolysis	≥ 0.6 to 3.0 g/L	Reddish
Very hemolysis	≥ 3.0 g/L	Reddish to Brownish

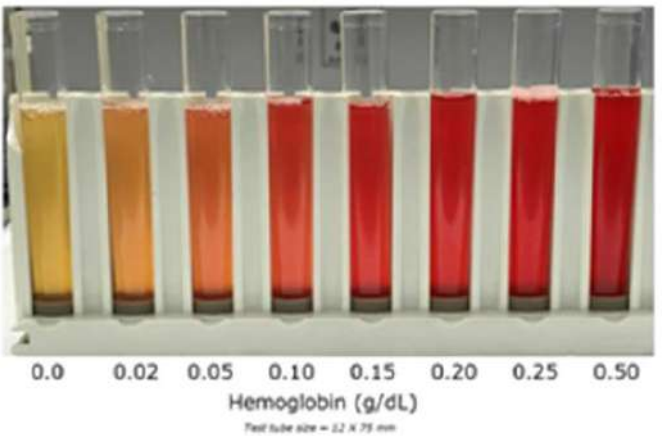


Figure 1. Plasma appearance with various hemoglobin levels. In the picture, with the higher levels of free hemoglobin in plasma or serum, the appearance of plasma or serum will be increasingly red [2].

Erythrocytes can also be destroyed in macrophages (extravascular hemolysis or hemolysis intracellular) of the mononuclear phagocytic system of the spleen, liver, and bone marrow. Extravascular hemolysis cannot cause hemoglobinemia and hemoglobinuria but it usually causes hemolytic jaundice (hyperbilirubinemia associated with bilirubinuria) [4].

2.1. *In Vivo* Hemolysis

In vivo hemolysis can be divided based on where the damage to red blood cells occurs. Intravascular hemolysis occurs when the damage of erythrocytes is still in the vascular system, whereas extravascular hemolysis occurs when the damage of erythrocytes occur due to the phagocytic system in the liver, spleen and bone marrow [1].

2.1.1. Intravascular Hemolysis

Intravascular hemolysis occurs because of the rupture or lysis of red blood cells inside the circulation, which is known as red blood cells lysis in vivo. When erythrocyte membranes rupture, it releases the hemoglobin into

the plasma. If the concentration of hemoglobin is more than 20mg/dL it can cause visible change in plasma color (pinkish to dark-reddish, depends on how much the hemoglobin concentration), hemoglobinemia is the example of intravascular hemolysis [4].

After hemoglobin is released into the plasma, free hemoglobin (which is a tetramer) breaks down into hemoglobin dimers in plasma and there will be two mechanisms:

1. The circulating hemoglobin dimer is oxidized to methemoglobin, which is released into free heme and globin chains. Free oxidized heme (met-heme) binds to hemopexin (β-globulin, Hpx) and the metheme and hemopexin complex (met-heme/Hpx) is taken by receptors on hepatocytes and macrophages in the spleen, liver and bone marrow. Similarly, the hemoglobin/haptoglobin complex is taken up by hepatocytes and macrophages (in lower level). In these cells, hemoglobin is released into heme and globin chains. Globin is broken down into amino acids, which are then used for protein synthesis. Heme is oxidized by heme oxygenase forming biliverdin and releasing iron. Iron is transferred to apotransferrin (iron-carrying protein) in plasma or can be stored in cell as ferritin (i.e., iron bound to the storage protein, apoferritin). The remaining porphyrin ring (biliverdin) is degraded to unconjugated bilirubin by biliverdin reductase. If the hemoglobin/haptoglobin complex is internalized by macrophages, the unconjugated bilirubin is released into the plasma, where it binds to albumin (to make it soluble in water) and is taken up by hepatocytes through the haptoglobin receptor [1, 5].
2. When hemoglobin exceeds the haptoglobin (this occurs at around the concentration of hemoglobin is 150 mg/dL), excess hemoglobin dimer can be filtered through glomerulus (the size of free hemoglobin is quite small, because the hemoglobin monomer is around 17 kD, far below the glomerular filtration threshold). This will cause hemoglobinuria and a positive result for heme on the dipstick (no erythrocytes seen in urine sediment and no evidence of

severe skeletal muscle injury causing myoglobinuria). Because the kidney tubules are able to take hemoglobin, it makes unconjugated bilirubin to be conjugated and released back into the urine, a positive reaction for bilirubin (indicating the presence of conjugated bilirubin) in the urine (i.e., bilirubinuria) (Figure 2) [1, 5].

Causes of intravascular hemolysis, include [1]:

a. Microangiopathic hemolytic anemia: thrombocytopenic purpura thrombotic, hemolytic uremic syndrome, disseminated intravascular coagulation.
b. Infectious agents: Malaria, Babesia, Bartonella bacilliformis, clostridial sepsis.
c. Paroxysmal nocturnal hemoglobinuria: Complement-mediated direct hemolysis.
d. Isoimmune hemolytic anemia: Transfusion reactions, hemolytic disease in newborns.
e. Mechanical damage due to prosthetic heart valves that are damaged or run for too long (hemoglobinuria).
f. Injury due to RBC caused by heat, chemicals, or poisons.

2.1.2. Extravascular Hemolysis

Extravascular hemolysis occurs when red blood cells are excreted by macrophages into the reticuloendothelial system (especially the spleen, but also by the liver and bone marrow). The example of extravascular hemolytic is autoimmune hemolytic anemia, where red blood cells are coated with antibodies. Spleen microphages detect antibodies, bound and phagocytosis and digest the red blood cells; this is done without any leakage of the RBC enzyme into the circulation, so that the inflammatory response (such as fever and cold) is usually mild. Many red blood cells are released and lose part of their cytoplasmic membrane, and then lose the central pallor and are seen as spherocytes in the blood smears. Patients with chronic extravascular hemolysis usually experience splenomegaly. Clinical symptoms with

abnormalities of extravascular hemolysis are generally milder and more stable than those seen with intravascular hemolysis, although cases of acute hemolytic crisis can occur [1, 5].

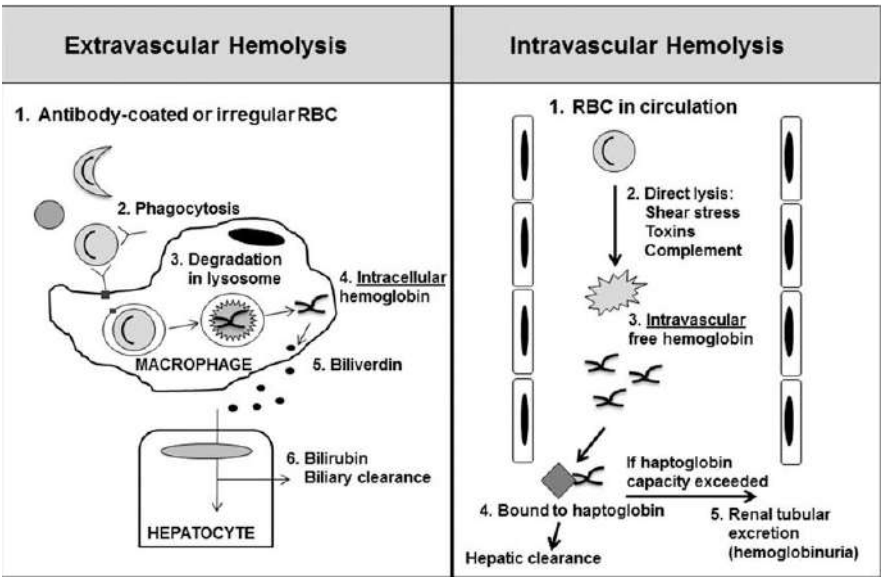


Figure 2. The difference of the mechanisms of extravascular and intravascular hemolysis [5].

Causes of extravascular hemolysis include [1]:

a. Autoimmune hemolytic anemia: positive DAT with severe case, free hemoglobin can circulate and this case resembles the intravascular hemolysis because the capacity of the macrophage system to process damaged red blood cells is exceeded.
b. Mostly hemoglobinopathy and thalassemia.
c. Hereditary spherocytosis, hereditary elliptocytosis.
d. Heinz body hemolytic anemia: G6PD deficiency, some drugs and poisons, Köln hemoglobin.

The most common red blood cell abnormalities that are seen on those causes are spherocytes, although target cells and sickle cells can be seen in hemoglobinopathies and Heinz body hemolytic anemias.

2.2. *In Vitro* Hemolysis

Poor sample quality is a source of errors that often occur in the pre-analytic phase. Among the samples that are submitted to the laboratory for testing that were considered not suitable for analysis, in vitro hemolysis accounted for about 40% - 70% of cases. In vitro hemolysis can occur at various stages including during the process of collecting blood, handling and processing of samples, and during the storage [2, 4].

In vitro hemolysis can cause a positive or negative bias in the analyte. The mechanism of bias in in vitro hemolysis includes proteolysis from analytes into intracellular compounds, release of thromboplastic substances, dilution of analytes due to release of cytoplasmic content, release of analytes themselves from erythrocytes, and analytical disorders due to hemoglobin and other intracellular substances. Interference that occurs can also be due to hemoglobin levels themselves, due to the spectral nature of this compound which has a peak absorbance at 420 nm and shows a significant absorbance between 340 and 440 nm and between 540 and 580 nm. In addition to spectral interference, hemoglobin also interferes with the activity of iron atoms, in which iron atoms participate in oxidation reduction reactions or in reactions involving hydrogen peroxidase [4].

The prevalence of in vitro hemolysis can vary depending on the number of patients, whether trained phlebotomists or unexperienced individuals in collecting samples, and whether samples will be directly processed or will be sent remotely for processing. With a significant time delay between picking and processing, the prevalence of in vitro hemolysis in outpatients has been found to be around 90 times less when compared to samples collected from patients in the Emergency Care Unit, where studies often show in vitro hemolysis in about 10% of samples from this location [4].

Table 2. The main cause of in vitro hemolysis [2, 4, 6]

Main cause	Specific
Patient's condition *(Patient-dependent)*	Fragile vein Improper vein access
Operator condition *(Operator-dependent)*	Operator skills Location of the needle syringe Blood drawn in a traumatic place Venous loss during venipuncture Stab in the hematoma area Intake of capillaries Antiseptics are used for the process of bleeding Placement of a long tourniquet
Device condition *(Device-dependent)*	Make a fist Underfilling of the tube Collection with inappropriate devices The use of the needle syringes Catheter and intravenous (IV) access Butterfly device Small needle Partial obstruction in IV line Containment by hand especially for drawing children's blood
Handling of the specimen	Inadequate homogenization Too much homogenization Transferring samples from the syringe to the vacutainer
Shipping of the specimen	Transportation through pneumatic tubes Significant time delays in specimen transport Conditions of transportation (mechanical trauma, time, temperature and humidity) Contact the jar directly with ice gel or ice.
Processing of the specimen	Significant time lag between receiving the specimen and centrifugation Extreme centrifugation temperatures Speed of centrifugation Repeated centrifugation of specimens that have been previously centrifuged
Storing of the specimen	Improper storage temperature before analysis Storage time before analysis

2.2.1. The Incompatibility Procedure in Specimen Collection

Blood cells are generally vulnerable and fragile, red blood cells can rupture when exposed to a mechanical trauma, osmotic shock (exposure to nonisotonic fluid), and extreme temperatures (e.g., during transportation to the laboratory). Excessive aspiration can cause red blood cells to be completely destroyed on the way as it passes to the syringe because of turbulence and physical strength. Hemolysis is more likely to occur when a patient's blood vessels are difficult to find, blood vessel are collapsed and the blood is removed by a syringe or vacuum tube, or when excess pressure is maintained during collection. Thus, experience and use of appropriate techniques by a phlebotomist or nurse is the key to preventing hemolysis [2].

The remaining wet antiseptic alcohol before venipuncture can cause red blood cells to rupture. The use of the wrong type of tube can cause hemolysis due to chemical or physical action, especially in some physiopathological situations. Underfilling/partial tube filling, which may occur rather frequently during blood taking, is often overlooked as a potential cause of hemolysis [2].

Venous stasis is often ignored as a source of preanalytic variability in test results which can cause various kinds of interference or interference in laboratory results. In particular, in the use of a tourniquet for too long (eg more than 3 to 5 minutes), the effects of venous stasis can cause a discharge of water, ion diffusion, and small molecular substances into blood vessels thereby increasing the concentration of various blood analytes in punctured locations and affecting test results. Moreover, when the vascular microenvironment experiences hypoxia and stasis, bioproducts such as protons will accumulate, and this has the potential to cause changes in laboratory parameters. Hemolysis in the specimen was systematically assessed by measuring the hemolysis index (HI) in the modular Rochesystem and a threshold corresponding to 48 g/L of free serum hemoglobin is used to classify the sample as hemolysis. The reason for hemolysis in the duration of venous stasis is still unknown. Possibly because of the occurrence of hemoconcentration and changes in water balance in cells, lysis of red blood cells and platelets may occur. When a tourniquet is placed for too long, hematoma can also occur. Clenching hands repeatedly

with or without the application of a tourniquet can also cause damage to red blood cells and thus produce hemolytic blood specimens [2, 4, 6].

2.2.2. Blood Collection with Various Devices and Needles

The blood specimen collection system consists of the use of double-ended needles, plastic holders or adapters and a series of vacuum tubes with rubber plugs. The blood collection procedure with this device seems to produce the best quality blood samples for laboratory testing and contextually ensures a greater level of safety for the operator because blood flows directly to the appropriate blood collection tube without external contact or requirements for additional syringes. One of the main problems that involve specimen hemolysis problems is inappropriate sampling, such as intravenous catheters (IV), butterfly devices, and small needles [2, 4].

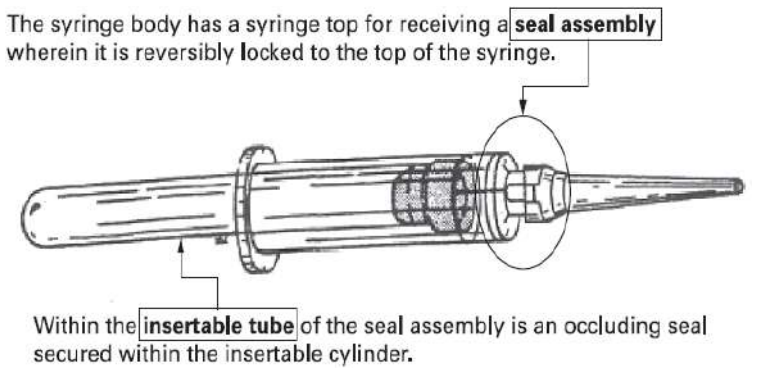


Figure 3. Vacuum specimen collection tube [2].

2.2.3. Specimen Collection with Syringe

The advantage of using a syringe is that the operator can control the pressure that will be given when taking blood. However, the use of syringes compared to the use of a vacuum tube system is not recommended because economically, it requires additional costs for syringes and safety reasons (risk of being pricked when transferring to a blood collection tube). In a study conducted by Becton Dickinson Diagnostics, hemolysis was visually observed in 19% of patients who took specimens using syringes and only 3% had hemolysis with vacuum tubes. This was also proven by other studies,

namely Carraro et al's research, showing that most hemolytic specimens were caused by blood drawn too hard through the syringe (30.7% of cases), wing needle into the syringe (20.0% of cases), catheters IV into the syringe (16.5% of cases), and access of infusion into the syringe (11.5% of cases). Grant also observed that samples were more likely to experience hemolysis when collected with a syringe than with an evacuated tube system (9% vs 3%) [2, 4, 6].

Basically, transferring blood from the syringe to the blood tube can trigger red blood cells' injury and rupture, because excessive force applied to the booster during blood collection causes blood to enter the tube by force. Blood can also freeze or clot and undergo hemolysis when taken by large volume syringes, and the forcible transfer of blood from the syringe into a blood collection tube can damage the RBC membrane [2, 4].

2.2.4. Blood Drawing from Intravenous Catheter

When drawing blood from intravenous catheters, it is often known that the peripheral IV catheters are not full (there is a large air space in the blood collection system into the tube). or hemolysis. This mechanism can cause damage to red blood cells during blood collection with this device due to the biaxial pressure carried out so that the strain occurs at a certain time in critical areas. Additional causes of hemolysis are blood flow through various internal diameters (catheters and connectors) and at various angles; hence, turbulence can cause rupture of red blood cells [2].

It has been previously reported that the prevalence of hemolytic specimens may range between 15% and 25% when catheter diameters from 22 to 20-gauge are used compared to 3.8% in samples collected by conventional 21-gauge needle sizes.

A recent meta-analysis took eight observational, descriptive, comparative, and experimental studies to compare in vitro hemolysis between specimens collected from IV catheters and those collected from usual venipuncture (in most of these hemolysis studies assessed through visual inspection to detect changes in color indicating the presence of hemoglobin; in a small number of studies, hemolysis was measured by a spectrophotometer). The results of this study indicate that hemolysis varies

greatly between methods and units, occurring in 3.3% to 77% of blood samples obtained through IV catheters, whereas it occurs in only 0% to 3.8% of samples obtained through regular venipuncture. Hemolysis samples are obtained from IV catheters through different methods also ranged from 5.6% to 77% for samples obtained by vacuum tubes, 3.3% to 49% for samples obtained with syringes, 12.8% to 49% for samples obtained from new IV catheters, and 24% for samples obtained from catheter IV [2, 4].

Factors that contribute to in vitro hemolysis can be classified as anatomic/physiologic, related to equipment, or technical. The first category includes collecting specimens in the right arm (e.g., collecting blood from the dominant side of the patient's body may be easier), forearm, or antecubital space. Sampling in a distal vein section can also cause more hemolysis compared to the antecubital fossa because of the smaller venous diameter, slower flow, and increased resistance. Factors associated with equipment include plastic, smaller size, and new IV catheters; use of vacuum tubes or syringes; partial obstruction of the catheter; and size of the primary tube. Partial catheter obstruction is considered to increase the strength of aspiration when the syringe is used to collect samples. There are several technical factors, including difficult catheter placement, several attempts to place an IV catheter, a tube filled with less than half the full capacity, and excessive force to draw blood while filling the tube. Prolonged tourniquet placement is an additional cause of hemolysis due to increased pressure and extravasation of fluid into the intracellular space [2, 4].

Therefore, the collection of laboratory specimens from peripheral IV catheters is not recommended. In accordance with this consideration, a study of Burns and Yoshikawa identified a widespread practice in Japan of collecting blood from plastic catheters connected to patients (aiming to reduce the amount of puncture) as a major problem causing hemolysis in the ER. Specifically, a comprehensive analysis of the data showed that the main factors contributing to false hemolysis included taking blood from the distal arm versus the antecubital fossa ($p = 0.0054$), use of small-sized plastic (e.g., 22-gauge) versus larger plastic (e.g., 20-gauge) cannula ($p = 0.010$), a collection of less than half of a full tube versus greater than or equal to half of a full tube ($p = 0.016$), tourniquet placement extended to> 2 minutes

versus ≤2 minutes (p = 0.016), and the use of plastic versus metal cannula (p = 0.016). The collection of blood by syringe produces the same level of hemolysis that was observed by using an evacuated tube system (p = 1.00) [2].

Hemolysis rates vary among studies, but hemolysis occurs at very low levels when blood samples are collected via venous function (0% - 3.8%) compared with those obtained through IV catheters (3.3% to 77%). The main factors associated with hemolysis are (a) anatomical - venous punction in the right hand, arm, antecubital or smaller distal vein - and physiological, (b) equipment factor - plastic, smaller, and new IV catheter, barrier to larger size laboratory tubes; and (c) technical factors - difficult catheter placement, difficulty collecting blood, multiple or unsuccessful attempts to place an IV catheter, partial filling of the main vacuum tube and excessive strength in blood aspiration or filling syringes [4, 6].

2.2.5. Drawing Blood with Small Needle Size

Blood collection is usually done with needles ranging from 19 to 25 G. Needle sizes from 19G to 21

G are used mainly for large antecubital veins. 23G needles are for small antecubitals, medium size arms, hand veins and 25-gauge or smaller needles are used for the smallest veins or in newborns and children. There are no guidelines on the use of needle sizes to be used to collect venous blood samples. Theoretically, blood must be drawn with a suitable needle to avoid excessive pressure or pressure shifting, which may eventually cause damage or rupture of blood cells, especially erythrocytes, producing unsuitable samples due to lumps or hemolysis. Use of the small needle has long been abandoned on the basis of some evidence that a needle equal to or smaller than 23 gauge may introduce a high degree of pre analytic variability, which can ultimately affect the reliability of test results. The use of needles that are too large (e.g., > 18-gauge) may also produce hemolysis for the possibility of greater venous disturbance because blood may enter the tube faster and stronger. Therefore blood aspiration through size standards (e.g., between sizes 19 and 21) is recommended; this causes less hemolysis than the use of

larger gauges because the flow rate, flow velocity, and turbulence are substantially reduced [2, 4, 6].

2.2.6. Collecting Blood Samples by Using a Spring Lancet or Manual

Skin puncture or capillary blood collection basically requires a puncture in the dermis layer of the skin to access capillaries that run through the subcutaneous layer of the skin. Blood obtained through this method is a mixture of unknown origins, where blood may be from arterioles, venules, capillaries, and added interstitial and intracellular fluid. However, the proportion of arterial blood is greater than venous blood, due to increased pressure in the arterioles leading to capillaries versus the exiting pressure in the capillary venules [2].

Collecting blood samples using a lancet is a minimally invasive and easily accessible procedure for obtaining blood samples, especially in newborns. CLSI capillary blood sampling via the heel is recommended for infants less than 1 year old, whereas capillary blood sampling from the finger joint is considered in children over 1 year old. Therefore capillary blood sampling is indicated for tests whose source is acceptable (for example, for Point-of-Care Testing [POCT]) when the required sample volume is relatively small; in patients with fragile, superficial, or difficult vein access; in cases where some venipunctures fail, especially if the test requires only a small volume of blood; in people with burns or scars in the collection of venous blood, when the patient is very obese; and in patients who need frequent blood tests, are currently receiving IV therapy on both arms or hands, are at risk for serious complications associated with venipuncture, venous thrombosis, or deep venous leakage (for example, deep venous puncture in infants or patients with thrombophlebitis); and chemotherapy. In contrast, capillary blood collection is not appropriate in patients who are severely dehydrated or those who are poor in circulation, for coagulation studies that require plasma specimens and tests that require large blood volumes (i.e., erythrocyte sedimentation rates [ESR] and blood cultures [2].

Several factors must be carefully considered before choosing the type of lancet and the location of the skin prick, including the age of the patient, the accessibility of the puncture location, and the volume of blood to be

collected. Another thing to be considered is identifying warm stab locations, which must also be pink and free of any calluses, burns, scars, bruises, or rashes (for example, it should not be cyanotic, edematous, or infected). Areas of the skin that show evidence of previous pricking should be carefully avoided [2].

Hemolysis that occurs during capillary blood sampling has been linked to various causes, including excessive squeezing, which induces an increase in hydraulic pressure in the capillaries; manual Lancet use, where the incision depth is not control ESR; mechanical fragility of the blood of newborns; and also high hematocrit values in newborns [2].

2.2.7. Transporting and Storing the Sample

In an original study on sending blood specimens from the peripheral collection center to the core laboratory by land vehicles for almost 8 hours, it was shown that the recommended temperature for transportation (i.e., between 2°C and 8°C) could be maintained only for a rather limited time (for example, up to 90 minutes), because internal temperatures are substantially dependent on the prevailing environmental conditions (i.e., external temperatures between 36°C and 38°C). In vitro hemolysis in samples can occur in a long-distance blood transportation because prolonged contact of serum or plasma with cells can trigger hemolysis. Additional causes include trauma induced mechanically during transport, which occurs during the handling of the specimen is transported several times (for example, due to a distant collection center and intermediate sampling or transportation sorting) [2, 4, 6].

For the purpose of speeding up the specimen delivery process, pneumatic tube systems (PTSs) enable fast and cost-effective delivery of patient specimens to the Clinical Laboratory and are therefore widely used in modern health centers. Depending on the configuration and speed of the system, samples transported through PTS can experience strong agitation due to sudden acceleration and deceleration along the trajectory, which results in the potential modification of various laboratory parameters [2, 4].

In vitro hemolysis in blood samples can also occur due to prolonged storage or in inappropriate conditions (for example, too hot or too cold).

Specifically, when serum or plasma is in contact with RBCs for a long time, hemolysis can take place. Placing blood tubes in direct contact with ice packs or frozen gels can also produce hemolysis.

The causes of hemolysis of the use of a pneumatic system are due to the high speed of transportation, sudden changes in container direction, and the pressure on the sample, caused by the system. Therefore, it needs a thorough monitoring of blood samples transported by the pneumatic tube system. In the installation of a pneumatic tube system, sudden changes in direction can be avoided.

In addition, system speed and pressure can be measured periodically and optimized to minimize the hemolysis of blood samples. Hemolysis can be prevented by using a gel in a test tube for blood collection. In addition, conveyor containers can be coated with cotton and protective circular rings to prevent hemolysis by minimizing acceleration and deceleration during transportation [2, 4].

2.3. How to Distinguish *In Vivo* and *In Vitro* Hemolysis

One of the most important problems faced by laboratories with hemolysis specimens is differentiating in vitro and in vivo hemolysis, a strategy that must be guided by clinical conditions rather than just analytical considerations. Currently, there is no consensus about how to help clinicians differentiate between in vitro and in vivo hemolysis, and clinical supervision is needed. When multiple samples are received by the laboratory, comparing a patient's hemolytic sample with another sample from the same patient received at the same time can help differentiate in vitro and in vivo hemolysis. For example, if the first sample is hemolysis but the second or previous sample is not, it heightens suspicion of hemolysis in vitro. On the other hand, if the first sample is hemolysis and the next sample also undergoes hemolysis, the probable cause is hemolysis in vivo, and a clinical history is needed to support this hypothesis [2, 4, 7].

Plasma depletion of haptoglobin has been traditionally considered as a reliable marker for the rapid identification for in vivo hemolysis. Varying with other potential markers, the haptoglobin level is not affected by in vitro hemolysis because the haptoglobin-hemoglobin complex is formed due to RBC damage in circulation which is quickly cleared by monocytes and macrophage tissue through the CD163 receptor. This parameter is available in most chemical analyses and most tools to distinguish between in vivo and in vitro hemolysis. Signs of in vivo hemolysis are an indirect increase in the bilirubin level and reticulocyte count, which is an indicator of the marrow compensation response. In in vitro hemolysis, reticulocyte counts remain normal. Increased LD H activity is typical of intravascular hemolysis [2, 4, 7].

3. The Effect of the Hemolysis Sample on Hematological Examination

It takes a small amount of red blood cells (RBC) to produce a visible plasma HGB. Unlike potassium and lactate dehydrogenase (LDH) levels, CBC results will show small changes with minimal hemolysis levels (≤0.05 g/dL HGB). As shown in Table 3 below (using % lysis to extrapolate% reduction in hematocrit [HCT]), with mild hemolysis at the level of 0.05 g/dL, HCT will decrease by about 0.2, which is within the range of analytic variations. Another thing to note is that to increase the average concentration of white blood cell hemoglobin (MCHC) from 34.0 to 36.0, severe hemolysis is needed (>0.7 g/dL free HGB), which shows that the majority of cases of mild to moderate hemolysis are not routinely detected by MCHC monitoring and will be reported without detection unless hemolysis is recorded during other tests [1].

Table 3. The Effect of a Hemolysis Sample on Hematocrit and MCHC in Two Different Levels of Hemoglobin [1]

					Changes Due to Lysis	
Plasma Color	Patient Hemoglobin, g/dL	Free Hemoglobin, g/dL	Lysis, %		Patient Hematocrit, %	Patient MCHC
Yellow	14.0	0.00	0.0		41.2	34.0
Orange	14.0	0.05	0.4		41.0	34.1
Red	14.0	0.15	1.1		40.7	34.4
Very dark red	14.0	0.78	5.6		38.9	36.0
Yellow	7.0	0.00	0.0		20.6	34.0
Orange	7.0	0.05	0.8		20.4	34.1
Red	7.0	0.15	2.2		20.1	34.4
Very dark red	7.0	0.78	10.6		18.4	36.0

3.1. The Effect of a Hemolysis Sample on CBC Results

Hemolysis results in a decrease in the number of RBCs and HCT values due to lysis. Hematological instruments usually lyse the sample before measuring the HGB, the number of PLT, the number of WBC, and the number of WBC differential cells, so these values are usually not affected by hemolysis. However, if hemolysis occurs due to pre-analytic variables, all the results are potentially unreliable [1, 3].

Because hematologic samples are not centrifuged before testing, hemolysis is usually detected from the following findings:

1. CBC is run, and an unexplained high MCHC value is found;
    - Because MCHC is calculated using the formula (HGB × 100)/HCT), where false low HCT is divided into HGB and will accurately produce high false MCHC. The normal range for MCHC will vary depending on the analyzer and must be determined for each laboratory. However, the typical level that triggers values ranges from more than 36.0% to more than 37.0%. The average white blood cell hemoglobin concentration

value at 37.0% is close to the solubility value for HGB, and further increase can cause crystallization. MCHC functions as a very useful index for detecting disorders, and although there is no hematologic analyzer that reports the hemolysis index, almost all MCHC reports [1].

- It is often recommended by instrument vendors to set a high MCHC review rate to minimize cases that require review. However, this will result in a significant loss of abnormalities, including hyperlipidemia and jaundice, warm and cold agglutinins, hereditary spherocytosis with true hyperchromic red blood cells, and hemolysis samples. Hereditary spherocytosis is the most common congenital RBC defect that leads to hemolysis, and skipping diagnosis can have serious consequences for patients. A conservative and empirical approach is to review cases that are above the upper MCHC reference range determined from normal local studies using the 3-SD range for MCHC. Because the 3-SD range covers 99.7% of the normal sample, 0.15% or 1 to 2 cases in 1000 normal results are expected to fall above that range. The level of laboratory reviews can then be adjusted upward if excessive numbers of false-positive samples (high MCHC for no reason identified) occur relative to the number of abnormal results that are actually found [1].

2. The CBC is run, and histogram abnormalities occur, triggering a sign on the instrument (e.g., RBC fragment, RBC abnormal scattergram). In practice, these flags are rarely seen in most analyzes and are not sufficient as the sole filter for hemolysis. When found, slide review for correct red blood cell fragmentation (schistocytes) and plasma examination for appropriate hemolysis are suggested [1, 3, 8].

3.1.1. Algorithm Approach for Evaluating High MCHC

When evaluating the hemolysis, other interference needs to be noted. Procedures must be adjusted for each laboratory to reflect medical needs,

instruments available for backup testing, and other practical considerations [1].

1. If the RBC is low and the average cell volume (MCV) is high, consider warm or cold agglutinins.
    - Prepare slides to look for a microscopic agglutination.
    - Warm the specimen at 37°C for 20 minutes, then mix and run again immediately. If MCV and MCHC fall and RBC increases, cold agglutinin is recommended. If the slide review supports cold agglutinin (visible agglutination without polychromasia or anemia), report warm results with comments (for example, "Correction results for cold agglutinin by heating").

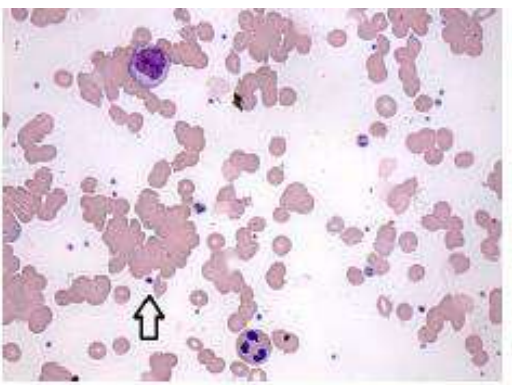


Figure 4. The arrows represent cold agglutination. With a picture like a collection of red blood cells but has unclear boundaries [1].

- If the slide shows agglutination that cannot be reversed on heating, automatic RBC, HCT, and index will not be reliable. If possible, do a spin micro-HCT and calculate an improved MCHC. Report with comments (e.g., "Cannot determine RBC, MCV, and MCH because of RBC agglutination which cannot be reversed at 37°C"). If a spinning HCT is not available, the HGB must be the only RBC parameter reported, with comments such as the following: "Cannot determine RBC parameters other

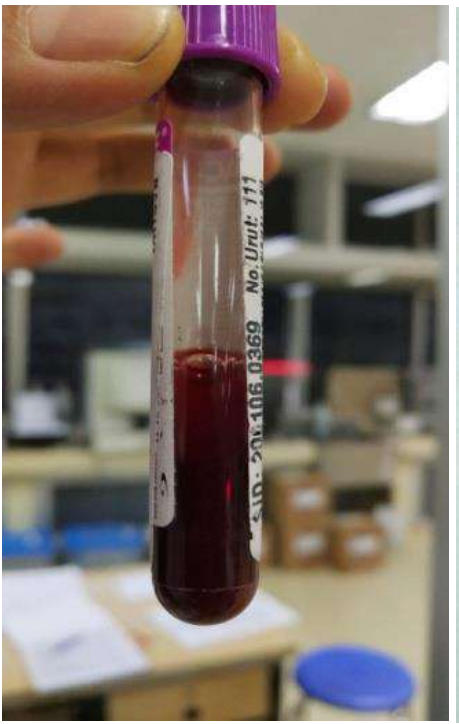
than HGB because agglutinin correcting is incomplete with heating" [1].

There are three reasons for the failure of agglutination after heating, including:

- Cold agglutinin that reacts strongly with high thermal amplitude. These patients do not have in vivo hemolysis, and red blood cell morphology will be normal without severe anemia and without a marked increase in polychromasia or a marked reticulocyte count. However, after the sample is cooled to room temperature, the RBC will only show partial disaggregation after heating, and the high MCHC will survive when the warm sample is retested.
- Cryoglobulin (antibodies precipitated by cold) is a rare cause of plasma turbidity in refrigerated samples. Such samples may have high MCHC which becomes normal after heating. In such cases, amorphous protein granules may be seen on Wright-colored slides made before heating the sample and centrifuged plasma will show turbidity because the protein will form a pellet at the bottom of the tube by centrifugation (unlike turbidity due to lipids). Although the findings of cryoglobulin are not specific, these can indicate important underlying conditions (infection, autoimmune disease, or lymphoproliferative disorders), so they must be reported if detected [1].

The comparison of samples with different hemolysis levels shows a decrease in the number of red blood cells and the number of hematocrit and an increase in mean corpuscular concentration of hemoglobin and platelet counts in samples with high levels of hemolysis. According to the accepted clinical point of view, samples with high hemolysis levels exceeding the desired bias, present a decrease in red blood cell count, hematocrit and volume mean corpuscular, and an increase in red blood cell distribution, meaning corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet counts. However, samples with mild levels of

hemolysis showed only a slight increase in mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count [3, 8].



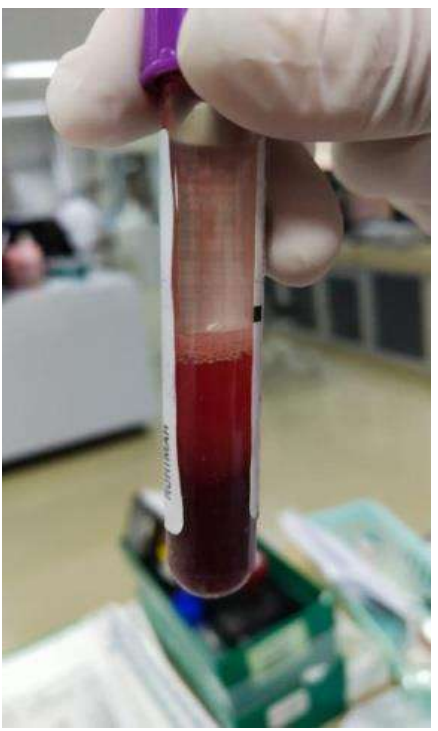
PARAMETERS		
HGB	12.3 *	[g/dL]
RBC	0.97 *	[10^6/uL]
HCT	9.2 *	[%]
MCV	94.8 *	[fL]
MCH	126.8 *	[pg]
MCHC	133.7 *	[g/dL]
RDW-SD	53.3	[fL]
RDW-CV	17.0 +	[%]
NRBC%	0.2	[%]
NRBC#	0.08	[10^3/uL]
WBC	48.96 +	[10^3/uL]
EO%	0.4	[%]
BASO%	0.1 *	[%]
NEUT%	7.8 *	[%]
LYMPH%	88.0 *	[%]
MONO%	3.7 *	[%]
EO#	0.22	[10^3/uL]
BASO#	0.05 *	[10^3/uL]
NEUT#	3.80 *	[10^3/uL]
LYMPH#	43.08 *	[10^3/uL]
MONO#	1.81 *	[10^3/uL]
IG%	0.5 *	[%]
IG#	0.25 *	[10^3/uL]
PLT	89	[10^3/uL]
PDW	7.9 -	[fL]
MPV	8.9 -	[fL]
P-LCR	13.9	[%]
PCT	0.08 -	[%]
IPF		[%]

a) b)

Figure 5. a) Blood sample with in vivo hemolysis b) In samples with in vivo hemolysis, the results of low HCT and calculation of RBC counts were also low. So the calculation of MCV, MCH and MCHC is high.

Dejonge, in his study, mentioned that the interpretation of CBC became disturbed in samples with severe degrees of hemolysis. The overall effect of hemolysis on complete blood count can be concluded. The value of white blood cells (WBC) is not affected, red blood cells (RBC) can be false low because the lysis of red blood cells and/or red blood cells in the form of fragments does not count as red blood cells. Hb calculations in hemolysis patients are usually accurate because red blood cells are fully lysis. Hematocrit levels become false low due to invalid calculations of MCV and false low red blood cells. The value of MCV (Mean Corpuscular Volume)

can be invalid i.e., false low or false high depending on the level of hemolysis. Some false lows are because red blood cell fragments produce a small pulse while in the diaphragm or false high because the fragments of red blood cells fall below the threshold of red blood cells so they are not counted. The MCH value becomes invalid due to a low false red blood cell value and the MCHC value becomes invalid due to a low false hematocrit value. RDW (Red Cell Distribution Width) is a false high because red blood cell fragments increase the CV (Corpuscular Volume) in the histogram. Platelet or PLT (Platelet) values can be false highs because erythrocyte fragments are read or counted as platelets [3, 8].



a)

PARAMETERS		
HGB	10.0 -	[g/dL]
RBC	3.90	[10^6/uL]
HCT	30.2 -	[%]
MCV	77.4 -	[fL]
MCH	25.6 -	[pg]
MCHC	33.1	[g/dL]
RDW-SD	46.8	[fL]
RDW-CV	16.6 +	[%]
NRBC%	0.0	[%]
NRBC#	0.00	[10^3/uL]
WBC	19.01 +	[10^3/uL]
EO%	0.1 -	[%]
BASO%	0.2 -	[%]
NEUT%	77.5 +	[%]
LYMPH%	16.9 -	[%]
MONO%	5.3	[%]
EO#	0.01	[10^3/uL]
BASO#	0.03	[10^3/uL]
NEUT#	14.75 +	[10^3/uL]
LYMPH#	3.22	[10^3/uL]
MONO#	1.00 +	[10^3/uL]
IG%	0.4	[%]
IG#	0.07	[10^3/uL]
PLT	540 +	[10^3/uL]
PDW	7.3 -	[fL]
MPV	7.6 -	[fL]
P-LCR	7.4 -	[%]
PCT	0.41 +	[%]

b)

Figure 6. a) Sample with in vitro hemolysis b) In samples with in vitro hemolysis, no abnormalities were obtained from the results of the examination. The results of CBC examination are influenced by how heavy the hemolysis.

The following picture is an illustration of patients undergoing hemolysis in which the data obtained from LIS RSUD Dr. Soetomo. The patient with a diagnosis of chronic lymphocytic leukemia, one of the causes of In Vivo hemolysis, where there is an abnormality in the histogram from CBC examination, namely a decrease in RBC and HCT values. In the histogram, erythrocyte flagging was obtained and there were also MCV, MCH and MCHC abnormalities. CBC examination was carried out at the Clinical Pathology Laboratory by Dr. Soetomo.

In Figure 6, a sample with hemolysis was also obtained, but the hemolysis was not as severe as in figure 5. CBC examination did not reveal any significant abnormalities in the histogram picture. The effect of CBC examination depends on the severity of the hemolysis that occurs.

3.2. The Effect of Hemolysis in Erytrhocyte Sedimen Rate (ESR) Test

The ESR measurement process is divided into 3 phases, namely formation of rouleaux, fast sedimentation and slow sedimentation. Factors that influence the ESR test are related to the erythrocyte number and mofrology which are the number of red blood cells, shape of red blood cells, agglutination of red blood cells, size of red blood cells and formation of rouleaux. In hemolysis, blood samples formed from red blood cells can be spherocytes or fragments depending on the cause of hemolysis itself. So the sample complicates the process of rouleaux which affects the ESR value. In samples with hemolysis, the ESR values will decrease. In the ESR test with the Westergreen method, it is often found that hemolysis samples are difficult to assess because of unclear boundaries [10].

The author also tries to check the ESR on an automated device, the alifax roller 20 LC, where the sample in Figure 5 yields a NF (Not Found) value and the sample in picture 6 is 42mm/hour. Then a manual ESR test was performed again on a hemolysis sample with a free hemoglobin level of 0.8 g/dL, the results were 3mm/hour and with an automatic examination the results obtained were 12 mm/hour.

a) b)

Figure 7. a) Blood samples are deliberately made hemolysis by spraying repeatedly using syringes b) The ESR test with hemolysis sample.

3.3. The Effect of Hemolysis in a Haemostatic Screening Test

Plasma prothrombin time (PPT) and partially activated thromboplastin time (APTT) is a routine coagulation test performed by the laboratory to evaluate the function of the coagulation system. The PPT test measures the extrinsic pathway, while the APTT measures the intrinsic pathway activity. Both coagulation functions are influenced by preanalytic factors such as venapungsi process, anticoagulant citrate dose, sample transportation, processing, and storage. Disorders of hemolysis, jaundice, and lipemia are the main problems in coagulation tests that use photo optic detection methods. Errors in the preanalytic and analysis phases can disrupt the reliability of the results [10, 11].

There are 2 main methods of measuring coagulation, the photo-optical method and the mechanical method. The optical method detects clot formation through optical changes and sample density (OD). The mechanical freezing method detects formation freezing by monitoring the movement of the steel ball in the test sample using a magnetic sensor. Hemolysis samples can cause interference with the photo-optical method. Therefore, samples with hemolysis cannot be examined for coagulation. According to the Clinical Institute and Laboratory Standards (CLSI), blood samples that show clear hemolysis can experience coagulation activity and also interfere with the detection of optical instrument clots [10, 11].

Laga et al. in their study explained their findings that PPT and APTT between hemolysis and non-hemolyzed blood samples did not differ significantly. Arora et al. argued that blood samples can be processed for coagulation tests because there is no significant difference between hemolysis and non-hemolyzed blood samples [10].

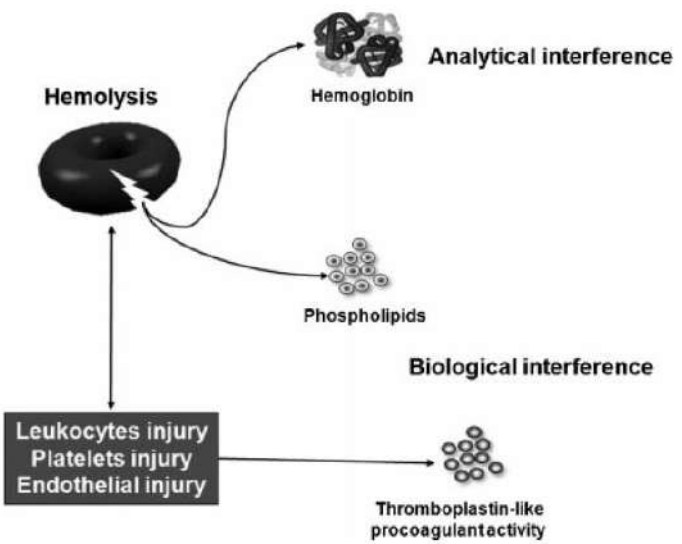


Figure 8. Hemolysis in Haemostatic Screening Test [11].

The effects of free hemoglobin produced by hemolysis can result in analytical and biological changes. High absorbance is caused by hemoglobin cells free from hemolysis which in the photo-optical method uses wavelengths. Cytoplasmic release and plasma membrane molecules (for example, tissue factors, proteases, phospholipids, and adenosine diphosphate) can falsely activate blood coagulation and platelets. The CLSI guidelines for PPT and APTT testing state: "samples with visible hemolysis should not be used because of the possibility of activating clotting factors and interference with end-point measurements" [10, 11].

Based on research conducted by Yetti Hernaningsih et al., namely comparing 2 groups (the first group is plasma hemoglobin level < 0.8 g/dL and the second group hemoglobin plasma level > 0.8 g/dL) with baseline. It was found that a weak positive correlation between plasma hemoglobin, PPT, and APTT. This is consistent with the findings by Laga et al., and Arora et al. That there is no significant difference in PPT values between non-hemolysis samples and samples obtained from healthy volunteers and mechanical in vitro hemolysis even when there are statistical differences found in patients, is very small, and not clinically significant. This is safe if the patient's value is still in the normal range. However, it will not be safe for patients with values at the upper or lower limits, because these values may have significant clinical implications [10].

Increased plasma hemoglobin levels cause the shortening of APTT. Some studies show different results for APTT values between samples that are not hemolysis and hemolysis which still cannot be clearly explained. This study is identical to the results from Lippi et al., in which APTT was shortened in hemolysis samples from patients with a normal baseline for APTT. Laga et al., and Arora et al., found shortening of APTT in hemolysis samples from patient subjects with normal baseline APTT and APTT extension in hemolysis samples from healthy volunteers using photo-optic instruments [10, 11].

The mechanism for shortening APTT in hemolysis samples has not been confirmed. This is thought to be caused by the release of phospholipids from erythrocytes and intracellular substances from leukocytes and platelets which can activate cascade coagulation. Other literature states that activating

the freezing cascade will cause PPT shortening and decreased fibrinogen levels, whereas APTT can be extended or shortened depending on whether there is activation or loss of fibrinogen. Hemolysis samples that are immediately examined may experience coagulation activation, so the APTT results are shortened. Meanwhile, hemolysis samples that are not directly checked and wait for some time will experience continuous activation, so that fibrinogen and coagulation factors are consumed more and this causes APTT extended results. Hemolysis sample testing in this study was carried out immediately after in vitro induction and all other procedures were carried out within 2 hours of phlebotomy. Reasons for APTT shortening in patients and prolongation of healthy volunteer APTT in previous studies were also related to lower factor VIIa levels in healthy subjects compared to patient subjects [10].

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Chapter 4

YOGA AS A PHYSICAL ACTIVITY AND CAM THERAPY IN THE MANAGEMENT OF OMINOUS OCTET IN TYPE 2 DIABETES MELLITUS: A COMPREHENSIVE SCIENTIFIC REVIEW

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ABSTRACT

Type 2 diabetes (T2DM) is a chronic metabolic disorder which significantly impacts health, quality of life and life expectancy. From triumvirate model to the egregious eleven model, our understanding on the pathophysiology of type 2 diabetes is ever expanding. The chronicity of diabetes mellitus, the involved direct and indirect costs, and adverse effects associated with the conventional medicines makes increasing number of patients turn towards complementary and alternate (CAM) therapies. Yoga

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is one such CAM therapy which is classified under mind-body medicine by the world health organisation (WHO).

The significant role played by physical exercise in the effective management and prevention of T2DM is well documented. The available scientific literature on the benefits of yoga clearly demonstrates that yoga is more than just a milder form of physical exercise. Beneficial effects of yoga, especially in reducing inflammation, oxidative stress, salivary cortisol, lipid profile, autonomic imbalance and glycemic control has been well documented. Inflammation and oxidative stress plays a central role in the underlying pathophysiology of diabetes (both insulin resistance & beta cell dysfunction) and its complications. One distinguishable advantage of using yoga over physical exercises is the relatively lower cardiovascular demand which makes yoga.

Apart from the common pathophysiological abnormality of insulin resistance (in muscles and liver) and beta cell dysfunction, the 'Ominous octet' model of DeFronzo refers to the involvement of brain, adipose tissues, gastrointestinal hormones, kidney and alpha cells in the pathophysiology of T2DM which requires multiple drug combination to rectify the underlying pathophysiological abnormalities, instead of simply trying to reduce the HbA1C levels. The current review is aimed at summarising the available research evidences on the effect of yoga in managing the 'Ominous octet' of T2DM and ensure evidence-based practise of therapeutic yoga in a clinical set up.

Keywords: CAM therapy, type 2 diabetes, ominous octet, physical activity, yoga

BACKGROUND

Yoga is a mind-body medicine widely accepted and practised across the world. Physical activity plays a major role as a part of the lifestyle interventions given to patients with type 2 diabetes (T2DM) [1, 2]. However, administration of physical activity has to be done with caution in T2DM patients with increased risk of microvascular and macrovascular changes, such as proliferative retinopathy and diabetic neuropathy which could possibly be aggravated by intense exercise regimens [3]. The beneficial effects of physical activity has been emphasized and in spite of its multiple known beneficial effects, adherence rate to physical activity is poor due to

various reasons. Complementary and Alternative Medicine (CAM) therapies are gaining increasing significance in the primary and secondary prevention of T2DM.

Yoga is claimed to be more a way of life, and just a mere low-to-moderate intensity physical activity and could act as a major replacement to patients with T2DM in whom exercise is contra-indicated due to the low cardiovascular demand [4]. In addition to the physical movements (*asanas*), yoga also includes controlled breathing (pranayama), relaxation, concentration (*dharana*), dhyana (*meditation*) and also involves social & self-discipline practices (*yama & niyama*) [5]. Therapeutic effects of yoga on various diseases are being documented, most of which are psycho-somatic and non-communicable disorders (NCDs). Of which, effect of yoga on T2DM has been widely studied and reported to have multiple benefits such as better glycemic control, improved lipid profile, cognition, weight reduction, nerve conduction velocity, reduced inflammation and oxidative stress, which plays a significant role in overcoming the risk factors contributing to the etio-pathogenesis of T2DM and its complications [6-8]. Comparative studies and systematic reviews have shown yoga to be as effective, if not superior to physical exercise in the management of cardiovascular risk factors associated with T2DM such as blood glucose, lipid profile, oxidative stress and cortisol levels [9, 10]. Yoga, in fact could possibly be beneficial in overcoming the 'Ominous octet' of T2DM and thus could be of greater benefit in the primary and secondary prevention of T2DM. The 'Ominous octet' model of DeFronzo [11] and yogic management of the same would be dealt in detail in the future sections.

YOGA AND THE OMINOUS OCTET

Beta Cell Dysfunction

β-cell dysfunction is one of the primary pathological abnormality observed in T2DM which classically involves three components- the secretion timing disorder, quantitative and qualitative disorder. Secretion

timing disorder in T2DM involves reduction in acute-phase insulin release (AIR). Over 70% of the AIR secretion is attributed to the autonomic nervous system [12]. AIR has been reported to be abolished by atropine in humans and by vagotomy in animal models [13, 14], suggesting the vagal involvement in AIR. While quantitative disorder involves increase or decrease in the second phase insulin release, qualitative disorder involves increase in the pro-insulin to insulin ratio [15]. Pro-insulin to insulin ratio is found to be increased in general in T2DM. An increase in the amount of circulating pro-insulin is an indication of impaired cleavage capacity of the beta cells which is independent of the duration of diabetes [15]. This has its own clinical significance, suggesting that beta cell dysfunction might possibly precede insulin resistance in the pathophysiology of T2DM and need not necessarily be secondary to insulin resistance.

Chronic oxidative stress and free radical damage are the key factors involved in beta cell dysfunction. Beta cells are more prone to oxidative damage due to the limited defence they possess against free radicals [16, 17]. The oxidative damage leads to do decreased transcription of insulin gene, protein misfolding and endoplasmic reticular (ER) stress in the beta cells [18].

Yoga studies in the past have reported a significant reduction in oxidative stress and increase in anti-oxidant defence with yoga, with improvement in various markers such as superoxide dismutase, glutathione and malondialdehyde [19, 20]. Likewise, γ-aminobutyric acid (GABA) which brings about membrane depolarization and proliferation in the beta cells were found to increase significantly with the practise of yoga, than walking [21, 22]. Similarly, the effect of yoga in improving vagal tone, as reported in previous studies, would be beneficial in overcoming the qualitative disorder of beta cell dysfunction, as well as the quantitative disorder via cholinergic stimulation of beta cells [23, 24]. Thus, yoga has the potential to overcome on multiple aspects of beta cell dysfunction in the pathogenesis of T2DM.

Insulin Resistance

Insulin resistance, along with beta cell dysfunction are the two common pathological abnormalities often acting as a therapeutic target for the management of T2DM. As skeletal muscles being the major target organ for utilisation of the ingested glucose in individuals with normal insulin sensitivity. Increase in the postprandial blood glucose levels leads to increase in insulin secretion which induces glucose uptake in skeletal muscles [25]. There are also insulin-independent mechanisms through which glucose is disposed of in skeletal muscles, via muscle contraction, hypoxia and nitric oxide through glucose transporter 4 (GLUT 4) translocation [26, 27]. T2DM characterised by impaired insulin-dependent glucose uptake, while insulin-independent glucose uptake via Adenosine 5'-monophosphate-activated protein kinase (AMPK) pathway still remains intact in the skeletal muscles of patient with T2DM [28].

Previous reports have shown yoga to improve insulin sensitivity both in normal healthy individuals and in T2DM [29, 30]. The observed increase in glucose sensitivity could be attributed to the possible activation of AMPK via the muscle contractions that occurs with asanas.

Adipose Tissue and Inflammation

T2DM is a chronic low grade inflammatory condition [31]. Adipocytes play a key role in regulating glucose homeostasis and adipokines in particular, play a major role in the etiopathogenesis of T2DM and adipocyte modulation could be an important treatment target for the management of T2DM [32].

Yoga studies have consistently shown to reduce pro-inflammatory markers such as interleukin 6 (IL-6), tumour necrosis factor α (TNF- α), C-reactive protein (CRP) and high-sensitivity CRP (hsCRP) and increase anti-inflammatory markers like adiponectin [33-35]. Adiponectin plays a major role like reducing hepatic glucose production (HGP) [36], increase in endothelial nitric oxide synthase (eNOS) which is beneficial in the

prevention of microvascular and macrovascular cardiac complications in T2DM [37, 38].

Brain

It has been documented that a lesion in the floor of the fourth-cerebral ventricle could induce diabetes way back in 1854 [39]. Evolutionary link between the neurons and beta cells is very much intact in various animals [40, 41]. Even in the higher animals, hypothalamus senses blood glucose levels very much similar to that of the beta cells [42].

High degree of association between brain and glucose metabolism has been well documented. There are many glucose sensing neurons in brain, hypothalamus in particular with these glucose sensing neurons (in arcuate, ventromedial and paraventricular nuclei) which helps in maintaining overall glucose homeostasis [39, 43]. Injecting smaller doses of insulin or glucose has shown to lower systemic blood glucose levels or increase liver insulin sensitivity, irrespective of the amount of insulin in circulation [44]. Administration of sub-minimal quantity of leptin in the third ventricle has shown to reverse insulin resistance and diabetes phenotypes in mouse model [45]. Similarly, brain derived neurotrophic factor (BDNF) and serotonin have shown to prevent beta cell apoptosis and increase beta cell proliferation respectively [46]. Yoga studies of the past have shown that yoga helps in increasing both BDNF and serotonin. Similarly, disturbances in the glucose metabolism also affect the brain. The risk of cognitive impairment increases by four-folds with HbA1C above 7% [47] and T2DM is associated with 1.5-2.5 fold increase in the risk of cognitive dysfunction [48, 49].

Bromocriptine is one of the glucose lowering drug used, which happens to be a sympatholytic D2-dopamine agonist [50]. The mechanism of action is through its effect on the dopaminergic activity in the brain and thereby reducing the sympathetic tone with subsequent increase in the parasympathetic tone and dopamine levels [51]. One of the important studies ever conducted in yoga has shown that meditation helps in cortical plasticity and regular practitioners of meditation were found to have increased cortical

thickness in areas associated with cognition and emotional processing [52]. Improvement in cognitive function has been observed in patients with T2DM who regularly practise yoga [53]. Increased grey matter in the limbic system, cerebral lobes and cerebellum, with increase in cerebral blood flow and activation of midbrain were some of the other benefits observed [54]. Yoga thus could play a vital role in the regulation of glucose homeostasis, possibly via hypothalamic-pituitary-adrenal (HPA) axis and potentially improve cognitive dysfunction in T2DM.

Glucagon

Importance of glucagon and α-cells was observed as early as 1947, when diabetes induced in animal model was ameliorated just by the removal of pancreatic alpha cells. T2DM is characterised by elevated glucagon levels and α-cell function [55]. A relatively recent study reported that even in complete deficiency of insulin as in type 1 diabetes (T1DM), blocking of glucagon action helps in reversal of metabolic and clinical derangements associated with T1DM in mouse model, which demonstrates the importance of glucagon suppression in the pathogenesis of diabetes [56]. Elevated plasma glucagon leads to increase in hepatic glucose production (HGP) and decreased insulin sensitivity in animal models [56, 57]. Similarly, the capacity of liver to synthesise triglycerides increases during stress which is partly attributed to role of glucagon through cyclic AMP pathway [58]. Likewise, GABA induces membrane hyperpolari-zation in the α-cells [59], which results in the glucagon suppression and as discussed above, yoga increases GABA levels [60, 61].

Glucagon-Like Peptide 1 (GLP-1)

Glucagon-like peptide 1 (GLP-1) is a potent incretin hormone, acting through multiple pathways such as increased insulin sensitivity in skeletal muscles and liver, delayed gastric emptying, inhibiting glucagon secretion,

promoting insulin and somatostatin secretion [62]. GLP-1 analogs is one of the most widely used treatment modality in the management of T2DM. In fact, the genes encoding GLP-1, known as the proglucagon gene, has three known sites of expression, namely α-cells, L cells of large intestine and nucleus tractus solitarius in brain, which also happens to be the nucleus of vagus [63]. Role of vagus nerve in the regulation of GLP-1 secretion has been clearly demonstrated through various animal studies. Bilateral sub diaphragmatic vagotomy in conjunction with gut transection and selective hepatic branch vagotomy completely abolishes the fat-induced and exogenous GIP induced GLP-1 release respectively, while stimulation of the distal end of the celiac branch of the sub diaphragmatic vagus nerve significantly increases the release of GLP-1 [64]. GLP-1 inhibits gastric emptying and acts via the vagal afferent mediated central mechanism [65], which could positively be influenced by yoga due to its known property of vagal activation. However, this is purely speculative based on the known effects of yoga on the autonomic nervous system and robust studies are needed to establish the impact of yoga specifically on GLP-1 secretion.

Liver

Glucose metabolism primarily occurs in the Liver and hepatic glucose production (HGP) is the major source of fasting hyperglycaemia, contributing to approximately 80% of diurnal hyperglycaemia in T2DM [66]. An increase in the circulating blood glucose levels releases insulin and inhibit hepatic glucose production, but this negative feedback is dysfunctional in T2DM. Increased flux of free fatty acids to liver and accumulation of liver fat are the major determinants of the decreased sensitivity of endogenous hepatic glucose production to insulin [67], Glucagon [56], central nutrient and hormone sensing in the hypothalamus together play a central role in regulating peripheral glucose homeostasis and mediate in reduction of hepatic glucose production via vagal nerve efferent signalling to the liver [68].

Various meta-analysis and systematic reviews on the effect of yoga on T2DM consistently report a higher reduction in the FPG (fasting plasma glucose) levels, than PPG (post-prandial plasma glucose) levels, which suggests a reduction in the hepatic glucose production [3, 69]. Although the effect of yoga on HGP has not been measured directly so far, one could speculate that the aforementioned positive impact of yoga on glucagon and adiponectin would have an influence in reducing the HGP. An improvement in the lipid profile through yoga is attributed to the increased hepatic lipase activity, which affects the lipoprotein metabolism and increases uptake of triglycerides by adipose tissue [61].

Glucose Reabsorption (Kidneys)

Kidneys plays an important role in regulating glucose homeostasis as well. Inhibition of renal glucose reabsorption is one of the novel and effective strategies in the management of T2DM [70]. β-cell dysfunction could upregulate sodium-glucose co-transporter 2 (SGLT2) protein in the kidney of patients with T2DM. Both SGLT2 and glucose transporter 2 (GLUT2) are expressed more in the proximal convoluted tubules cells of T2DM patients than in healthy individuals, resulting in elevated renal glucose uptake and further worsening of hyperglycaemia [71]. Hypothalamic pro-opio melanocortin (POMC) deficiency improves glucose tolerance in mouse models, by increasing glycosuria and reduced sympathetic nervous system (SNS) activity which is attributed to observed glycosuria and improved glucose tolerance [72]. Likewise, the SNS activity reducing property of yoga might also help reduce renal glucose reabsorption, facilitating improved glucose tolerance and better glycaemic control. This is obviously an area for future yoga research.

OTHER PHYSIOLOGICAL EFFECTS OF YOGA

Yoga has been reported to be as effective, or superior to physical exercises [6, 10, 73, 75] and the benefits thus, might not just be due to the physical movements involved in yoga. Y*oga nidra*, which is a yogic technique which involves complete body relaxation without any physical movements. A three month study exploring the efficacy of *yoga nidra* in patients with T2DM found a significant reduction in the blood glucose levels when compared to the control group [74]. The study is an indication that the reduction in blood glucose might not only be due to the physical movements involved, but also via possible reduction in the stress hormones or due to increased vagal activity [76]. Decreased vagal tone contributes to the etiopathogenesis of T2DM in multiple ways [12, 77]. Reduced breathing rate increases vagal activation and decrease the influence of sympathetic branch of the autonomic nervous system, measured by an increase in heart rate variability and baroreceptor sensitivity [77, 78]. Some studies report betterment of the complications associated with diabetes, like improved nerve conduction velocity [79], cognition [53] and Quality of Life (QoL) [80]. Two studies have also reported a reduction in the medication score and insulin intake of T2DM patients [81, 82], suggesting a possible improvement in β-cell dysfunction, insulin resistance or any of the pathological abnormalities in the above mentioned ominous octet, which was not observed with the control group on exercise-based lifestyle intervention [82]. Thus, yoga shows promising insights of becoming an effective complementary therapy in the prevention and management of T2DM [83].

CONCLUSION

Benefits of yoga in overcoming risk factors associated with the etiopathogenesis of T2DM is multiple, and thus, yoga could be used as a safer CAM therapy for the management of T2DM. Yoga would also be a

cost-effective intervention and also sustainable over the long run, which has its own significance, especially considering the chronic nature of T2DM.

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Chapter 5

CYSTATIN C: BENEFITS AND PRECAUTIONS AS A UNIVERSAL BIOMARKER

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ABSTRACT

Background

Cystatin C has gained more attention as a promising biomarker due to several advantages over creatinine that suffers from the blind range (does not increase until 50% of the kidney deteriorates). Cystatin C levels are influenced as soon as any mild defect in the kidney occurs. Several non-renal diseases influence cystatin C. Thus, providing additional prognostic value for this promising biomarker.

Objectives

1. Investigate the effects of age and gender on cystatin C levels. 2. Challenge the glomerular filtration rate equations for healthy cases. 3. Compare the values obtained from different glomerular filtration rate equations. 4. Evaluate the prognostic value of cystatin C for selected non-renal diseases.

Methods

Using cross-sectional analyses, we established the relationship between cystatin C levels and non-renal predictors. The quantification of cystatin C was performed by high-performance liquid chromatographic method, while for creatinine by a colorimetric enzymatic method.

Results

Statistical data confirmed a non-significant relationship concerning age, gender, or smokers among the recruited healthy samples. For the recruited patients suffering from diabetes, hyper- and hypothyroidism, and cardiac dysfunctions, an apparent increase in cystatin C levels was observed except for hypothyroidism patients in which a decrease in their cystatin C levels was observed.

Conclusion

Diabetes, thyroid, and cardiac dysfunctions showed to influence the levels of cystatin C in human blood. On the other hand, age, gender, and smoking habit didn't show to influence cystatin C levels. Therefore, cystatin C could be considered as a useful biomarker of the mentioned diseases, in turn, this requires extra precautions including the evaluation of several clinical conditions by physicians should cystatin C is considered as a renal biomarker.

Keywords: cystatin C, biomarker, renal failure, diabetes, thyroid, cardiovascular

ABBREVIATIONS

Asymp. Sig.:	Asymptotic significance
BLD:	Below limit of detection
BLQ:	Below limit of quantification
CC:	Cystatin C
CKD:	Chronic kidney disease
CK-MB:	Creatine kinase-muscle/brain
Cr:	Creatinine
eGFR:	Estimated glomerular filtration rate
GFR:	Glomerular filtration rate
HIV:	Human immunodeficiency virus
HPLC:	High-performance liquid chromatography
IRB:	Institutional review board
LDH:	Dehydrogenase
PENIA:	Particle enhanced nephelometric immunoassay
PET:	Particle enhanced turbidimetric method
p:	Probability of the observed data
TFA:	Trifluoracetic acid
U:	Mann-Whitney test statistics
W:	Wilcoxon
Z:	Distribution of data (calculated from U)

INTRODUCTION

Cystatin C (CC) is a cysteine protease inhibitor, positively charged, low molecular weight plasma protein consisting of 120 amino acid residues, freely filtered through the glomerulus [1-10]. CC is also an early indicator of renal failure, and superior to other markers, in which it changes before creatinine (Cr) becomes abnormal (Cr does not change until 50% of glomerular filtration rate (GFR) is reduced, or 50% of the kidney is damaged); accordingly, a therapeutic opportunity is going to be missed [2, 4, 11, 12]. It has been previously proved that CC does not depend on the non-renal factors such as muscle mass, weight, gender, age [1, 4, 5, 9, 10, 13-16]. However, several studies have reported that some non-renal factors do have a clear impact on CC levels, such as malignant diseases including metastatic melanoma, colorectal cancer, human immunodeficiency virus (HIV) infection, *in vitro* increase production of CC in the presence of glucocorticoids, uncontrolled thyroid diseases [17-19], diabetes [2, 13, 20-22], and sepsis, which is a systemic condition that causes both cardiac and renal dysfunction [23-27]. Recent studies have highlighted the possibility of developing an acute kidney injury after cardiac surgery, which in turn necessitates the availability of ideal biomarkers such as CC to evaluate the situation quickly [28].

Here, we investigated the effect of age and gender on CC levels. Besides, we envisaged to confirm the diagnostic ability of CC for the selected dysfunctions: diabetes, hypo-/hyperthyroidism and cardiac dysfunctions, consequently, its prognostic superiority over Cr.

Furthermore, utilizing our results, we compared the efficiency of the available estimated glomerular filtration rate (eGFR) equations as an overall renal index for healthy and pathological samples: Also, we investigated the validity of considering those equations as a diagnostic tool in healthy population. Statistical analyses were considered to investigate whether a relationship does present or not.

Due to several disadvantages of the available immunoassay methods; Particle enhanced nephelometric immunoassay (PENIA) [6], particle enhanced turbidimetric method (PET) [29] and enzyme-amplified single radial immunodiffusion [30], a new high-performance liquid chromatographic (HPLC) method has been developed and validated by our group for the quantification purpose of CC in human blood [31]. The reasearch was inspired by the previously published work for quantifying CC in human urine [32, 33]. There are two HPLC-Mass analytical methods for the determination of CC. However, one of them was used only for raw material [34], and the other one, had no real biological samples tested [35].

MATERIALS AND METHODS

CC protein (>96%) was purchased from BBI Solutions (UK), HPLC grade acetonitrile, methanol, 1-hexane sulfonic acid sodium salt, trifluoroacetic acid (TFA), and acetone were purchased from Merck (Germany).

SAMPLE SELECTION

Healthy samples were divided according to age, gender, and smoking habit. Accordingly, the statistical analyses were introduced for each group against healthy control samples as follows: different age intervals* within the same gender were tested with respect to each other, and likewise for males and females within the same age intervals, and for smokers and non-smoker within the same age and gender. The rest of the pathological samples were divided according to the disease, and compared with the healthy samples.

*Age intervals: refer to categories under healthy samples section.

BLOOD SAMPLES COLLECTION AND PROCESSING

Blood samples from multiple patients with a known history of certain diseases:renal failure, diabetes, thyroid hyper-/hypothyroidism, and cardiovascular, were recruited from a hospital into heparinized tubes and were kept cool in an icebox.

SAMPLE PREPARATION

Blood samples were centrifuged at (4400 rpm, 4°C) for 5 minutes. 2 mL of supernatant plasma solution was withdrawn in a test tube, and about 8 mL of cold acetone was added, then the resulting solution was mixed thoroughly by shaker for 20 minutes, then centrifugation at (4400 rpm, 4°C) for 30 minutes took place, and the precipitate was dissolved in a 4 mL of 0.05% TFA (v:v). Centrifugation was done at the same parameters for 30 minutes, and the supernatant was filtered through 0.45 µm Teflon filter before injecting into the HPLC system [31].

CHROMATOGRAPHIC CONDITIONS

Dionex HPLC system (Dionex, Germany) with a degasser, low-pressure gradient pump, column oven, an autosampler, a UV detector was used.

Data acquisition was performed with Chromeleon 7.2 SR2 software. A reversed-phase Ace C_8 (150 × 4.6 mm i.d., 5 µm) column (supported with a guard holder contains Ace 5 C_8 100A guard cartridge) was placed in the column oven at 25°C. Mobile phase A of 0.01 M 1-hexane sulfonic acid sodium salt in water plus 0.05% TFA, pH = 2.4 filtered through 0.45 µm Teflon filter and mobile phase B (acetonitrile: methanol: mobile phase A) (300: 300: 225, v:v:v), pH = 2.5 filtered through 0.45 µm Teflon filter, were pumped at a flow rate of 1.0 mL/min and used for the elution of CC utilizing a gradient program. Before each run, the column was equilibrated with 65% of mobile phase B for 3 minutes. The gradient was increased gradually to 80% in 3 minutes and then to 100% in 5 minutes lasted 2 minutes to complete the separation. Then, the gradient was decreased to the initial conditions of 65% in 0.5 minute. At 14 minutes, the HPLC system is ready for the next injection. The injection volume was 100 µL. The eluent was monitored at 224 nm, for detailed validation tests refer to our published work [31].

STATISTICAL WORK

IBM SPSS statistics software (version 1.0.0.1012) was utilized to perform all the statistical analyses.

As the distributional assumptions could not be made, a non-parametric test (Mann-Whitney test) was used to evaluate the data.

Supplemental data (Supplemental Tables A-1 through A-9).

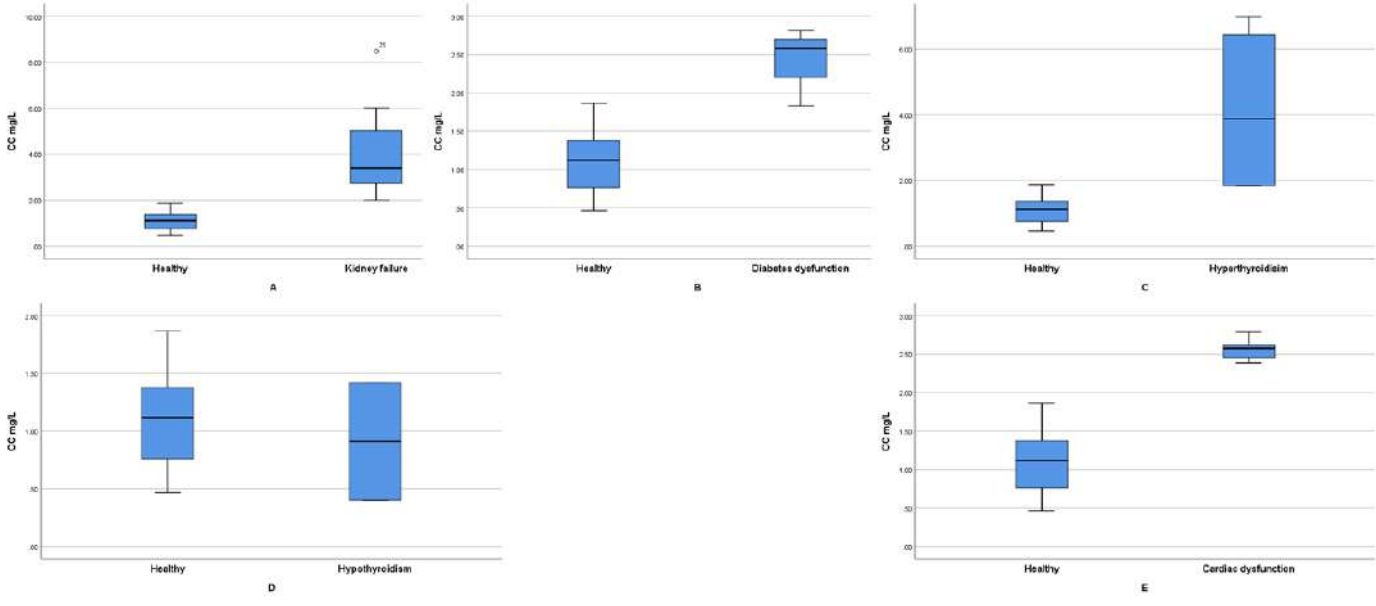

Figure 1. Boxplot graph of CC levels in various diseases *vs* healthy volunteers.
A. CC levels in kidney failure patients *vs* healthy volunteers: 27 samples, **(p < 0.001, Z = -5.532)**, highly significant relationship, reject null hypothesis. **B.** CC levels in diabetic patients *vs* healthy volunteers: 3 samples, **(p = 0.004, Z = -2.593)**, highly significant relationship, reject null hypothesis. **C.** CC levels in hyperthyroidism patients *vs* healthy volunteers: 4 samples, **(p = 0.001, Z = -2.866),** highly significant relationship, reject null hypothesis **D.** CC levels in hypothyroidism *vs* healthy volunteers: 2 samples, **(p = 0.655, Z = -0.531)**, non-significant relationship, retain null hypothesis. **E.** CC levels in cardiac patients *vs* healthy volunteers: (5 samples, **(p < 0.001, Z = -3.330)**, highly significant relationship, reject null hypothesis.

ETHICS STATEMENT

The study was approved by the institutional review board of King Fahad Medical City, Riyadh, Kingdom of Saudi Arabia (IRB review No. 17-051E, Category: Exempt).

Implied consent has been obtained from each volunteer.

RESULTS AND DISCUSSION

Healthy Samples

16 healthy volunteers were divided into three categories. Each category has two groups: i. non-smoker males age ≥ and < 50 years old, ii. Non-smoker females age ≥ and < 50 years old iii. Non-smoker males and females age ≥ 50 years old, iv. Non-smoker males and females age < 50 years old, v. Smoker and non-smoker males with age < 50 years old.

Slightly higher CC levels were observed in the subjects of ages ≥ 50 over those less than 50 (Supplemental Tables 1: a-c and 2: a-c), and it was more pronounced in males over females (Supplemental Tables 3: a-d) as well as in smokers over non-smokers (Supplemental Tables 4: a-c). The averages of each group were as follows: i. (1.41 and 1.11) mg/L CC, ii. (1.18 and 0.74) mg/L CC, iii. (1.15 and 1.00) mg/L CC. iv. (1.46 and 1.07) mg/L CC. However, by referring to the statistical data it was confirmed that those differences did not achieve the threshold of rejecting the null hypothesis, in which, the obtained p values for each category were (i. 0.400, ii. 0.667, iii. 1.000, iv. 0.178, v. 0.244). Consequently, the data above have confirmed a non-significant relationship between each category and CC levels. In conclusion, our work is in line with the studies that support the independence of CC on age, gender, and smoking habit [36, 37].

GFR is essential for diagnosing, staging, and management of chronic kidney disease (CKD). However, more studies need to be carried out to check the validity of the equations -in use- for non-CKD cases as well as

healthy subjects, bearing in mind that such equations were initially derived based on the data acquired from CKD patients. eGFR values for healthy population utilizing those equations sometimes underestimates GFR and consequently results in false-positive data. For example, in (Table 1, entry 7) CC level of 1.71 mg/L showed an eGFR value of 38.66 mL/min, which is classified as a stage 3 kidney failure! Such underestimation can also be noticed in many healthy samples that have been analyzed (Table 1, entries 4 to 11, 13, 15, and 16). As of yet, we can conclude that if the CKD - eGFR equations are used to calculate eGFR for the non-CKD patients, underestimated eGFR values (< 80 mL/minute) are expected despite the normal levels of CC as mentioned by Mussap M., et al., Shlipak., MG et al., and Johnson DW., et al. [2, 9, 38]. The same observations were noticed by Machado, J. D. et al. when they utilized those equations for healthy and diabetes patients [39].

Table 1. CC levels and eGFRCC for healthy samples

Entry	Gender	Age	mg/L	GFRCC
1	Male	41	0.73	118.34
2	Male	37	0.88	100.45
3	Male	33	0.48	151.13
4	Male	35	1.77	40.19
5	Male	34	1.87	37.64
6	Male	57	1.12	67.81
7	Male	57	1.71	38.66
8	Female	12	0.76	58.60
9	Female	42	0.72	7.28
10	Male	19	1.37	60.10
11	Male	20	1.19	72.05
12	Male	23	0.96	94.72
13	Male	8	1.42	60.18
14	Male	16	1.13	78.49
15	Male	43	1.08	74.75
16	Female	58	1.18	1.34
Min		8.0	0.47	1.34
Max		58.0	1.87	220.69
Average		37.0	1.11	75.44

Refer to Table 2 for eGFR equations

Table 2. eGFR equations

Equation basis and gender	Cr mg/dL	CC mg/L	eGFR equation
eGFRCC			
Female/Male		≤0.8	$133 \times (SCC/0.8)^{-0.499} \times 0.996^{Age}$ [× 0.932 if female]
Female/Male		>0.8	$133 \times (SCC/0.8)^{-1.328} \times 0.996^{Age}$ [× 0.932 if female]
eGFRCr			
Female	≤0.7		$144 \times (SCr/0.7)^{-0.329} \times 0.993^{Age}$ [× 1.159 if black]
Female	>0.7		$144 \times (SCr/0.7)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
Male	≤0.9		$141 \times (SCr/0.9)^{-0.411} \times 0.993^{Age}$ [× 1.159 if black]
Male	>0.9		$141 \times (SCr/0.9)^{-1.209} \times 0.993^{Age}$ [× 1.159 if black]
eGFRCr-CC			
Female	≤0.7	≤0.8	$130 \times (SCr/0.7)^{-0.248} \times (SCC/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$130 \times (SCr/0.7)^{-0.248} \times (SCC/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
	>0.7	≤0.8	$130 \times (SCr/0.7)^{-0.601} \times (SCC/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$130 \times (SCr/0.7)^{-0.601} \times (SCC/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
Male	≤0.9	≤0.8	$135 \times (SCr/0.9)^{-0.207} \times (SCC/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$135 \times (SCr/0.9)^{-0.207} \times (SCC/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]
	>0.9	≤0.8	$135 \times (SCr/0.9)^{-0.601} \times (SCC/0.8)^{-0.375} \times 0.995^{Age}$ [× 1.08 if black]
		>0.8	$135 \times (SCr/0.9)^{-0.601} \times (SCC/0.8)^{-0.711} \times 0.995^{Age}$ [× 1.08 if black]

Inker, L.A., et al. [40]

KIDNEY FAILURE PATIENTS

27 CKD patients, 13 female and 14 male with an age interval of 13 to 85 years old were included in the study. Noticeable increase in CC and Cr levels has been observed (2.00 to 8.48 with an average of 3.97, normal 0.47 to 1.87) mg/L and (1.46 to 6.83 with an average of 3.42, normal 0.84 to 1.21) mg/dL, respectively. Concurrently, a reduction in their eGFR values were also observed (8.94 to 52.58 with an average of 21.95, 0.04 to 34.71 with an average 9.58 and 8.80 to 32.65 with an average 16.42) mL/min utilizing eGFRCr, eGFRCC, and eGFRCr – CC formula, respectively. Statistical data of CC levels in the kidney failure patients *versus* healthy subjects showed a highly significant relationship ($p < 0.001$) (Figure 1) (Supplementary Tables 5: a and b).

GFR values have been established using the equations stated by Inker, L.A., et al. [40] (Table 2). It's been reported by the national kidney foundation that an eGFR equation that uses both serums Cr and CC with age, sex, and race is better than equations that use either of these two serum markers [40, 41]. Many studies have proved the superiority of CC marker, and it's better adequacy and accuracy in estimating eGFR than Cr [14, 42]. Moreover, it has been reported by Gharaibeh KA, et al. that CC can detect the renal recovery earlier than Cr [42]. The obtained eGFR values proved the superiority of CC as a renal failure biomarker, which is ascribed to its early prediction in response to any mild defect in renal functions. The overall relationship between the reciprocal levels of CC and GFRCC-Cr values was stronger than that in the case of Cr, $r^2 = 0.522$ and 0.370, respectively. Utilizing the Cr equation to calculate GFR might underestimate the severity of the CKD stage! For example, (Supplementary Table 5 a, entry 6) with an eGFRCr value of 30.62 mL/min will be classified under stage 3 (Moderate GFR decrease). However, it must be classified under stage 4 (Severe GFR decrease) based on eGFRCC and eGFRCr-CC values 17.58 and 21.80 mL/min, respectively. Nevertheless, in some cases, there might be no major differences in the final calculated eGFR values using any of these equations, and this is clear in (Supplementary Table 5 a, entries 3 and 7), in which, the obtained eGFR values were (10.51, 8.74, 8.8) and (30.62, 34.71, 31.38)

mL/min, (eGFRCr, eGFR CC, eGFRCr-CC, respectively). Statistical analyses showed significant differences between those equations (GFR CC to GFR Cr–CC), (GFR Cr to GFRCr–CC) (GFRCr– GFRCC) in CKD patients, $p = 0.008$, 0.046 and < 0.001, respectively. Significant relationships were observed between either eGFR Cr or eGFR CC and the reference eGFRCr-CC. However, it was higer in the case of eGFR CC ($p = 0.008$) versus ($p = 0.046$) for eGFR Cr, which is an advantage for CC over Cr as prognostic biomarker. The same observation was also reported by E. Wasén et al. and L. A. Stevens et al. [3, 41]. In conclusion, to minimize the errors in the final eGFR value, we recommend considering the equation that utilizes both serums. (Supplementary Tables 5: c-e).

DIABETIC PATIENTS

3 female patients with diabetes dysfunction with an age interval of 21 to 58 years were included in the study. Elevated CC levels were observed (1.83 to 2.82 with an average of 2.41, normal 0.47 to 1.87) mg/L. However, those patients have no recorded problems in their kidneys in addition to their normal Cr levels (0.49 to 0.84 with an average of 0.71, normal 0.84 to 1.21) mg/dL. Statistical data confirmed a highly significant relationship for the increased levels of CC in diabetic subjects, $p = 0.004$ (Figure 1) (Supplementary Tables 6: a and b). based on the obtained data, CC could be a promising biomarker to evaluate the involvement of diabetes in renal failure as also shown by previous studies.

THYROID PATIENTS

Thyroid dysfunction was proved to have a direct influence on CC levels. In which, elevated CC levels were observed in hyperthyroidism patients, and a reduction in hypothyroidism patients [18, 19]. To validate those studies, 9 patients with thyroid hypothyroidism and hyperthyroidism dysfunctions, 6

females and 1 male with an age interval of 28 to 61 years were included in the study. An observed increase in CC levels was observed in hyperthyroidism cases (1.83 to 6.98 with an average of 4.14, normal 0.47 to 1.87) mg/L, however, those patients don't have any recorded problem in their kidneys based on their Cr results, for some of them based on their history as we don't have their Cr results. From the statistical data, a significant relationship has been confirmed for the increased levels of CC in the hyperthyroidism subjects ($p = 0.001$) (Figure 1) (Supplementary Tables 7: a and b). On the other hand, a decrease in CC levels was observed in the hypothyroidism cases (0.40 to 1.42 with an average of 0.91, normal 0.47 to 1.87) mg/L. Furthermore, CC levels in 3 samples were even below the detection limit of the method (Supplementary Table 8 a, entries 2-4)[31]. However, a non-significant relationship was observed in the hypothyroidism data ($p = 0.655$) (Figure 1) (Supplementary Tables 8: a and b); thus, the null hypothesis could not be rejected. Such observation could be ascribed to the fact that the obtained CC levels in hypothyroidism patients fall in the healthy range. Nevertheless, we still have strong evidence that CC levels are depleted in hypothyroidism patients, in which we had 3 samples with very low CC levels with respect to the normal range. In conclusion, we confirmed that CC levels are influenced in the case of thyroid dysfunction, so CC can play a significant role in predicting thyroid dysfunctions as well. In turn, thyroid functions need to be considered when considering CC as a renal biomarker.

CARDIAC PATIENTS

5 patients with cardiac dysfunction, 1 female and 4 males with an age interval of 23 to 60 years were included in the study; those patients have a history of elevated rates of lactate dehydrogenase (LDH) as well as creatine kinase-muscle/brain (CK-MB) markers. An observed increase in their CC levels was observed (2.38 to 2.62 with an average of 2.51, normal 0.47 to 1.87) mg/L. However, those patients have no recorded problems in their kidneys, as can be observed from their normal Cr levels (0.83 to 1.02 with

an average of 0.95, normal 0.84 to 1.21) mg/dL. Statistical data confirmed a highly significant relationship for the increased levels of CC in cardiac dysfunction subjects, $p < 0.001$ (Figure 1) (Supplementary Tables 9: a and b). Accordingly, we proved that CC is significantly affected in patients with cardiac abnormalities. Consequently, CC can be considered as a marker for cardiovascular disease as well. The same conclusion was also stated by O'Neal, J. B. et al. [28].

CONCLUSION

Several studies have suggested the relationship between CC and other non-renal factors such as age, gender, smoking, among others. In our results, we confirmed that CC is independent of those factors. However, additional studies with more subjects are needed. Based on the data obtained from the comparison study among the three eGFR equations, we recommend using the equation that utilizes both markers as also recommended by the national kidney foundation. The superiority of CC as a marker of kidney failure has been confirmed. Furthermore, we have proved the effectiveness of CC as a useful biomarker of other diseases like diabetes, thyroid hyper-/hypothyroidism, and cardiovascular dysfunctions, in which an apparent effect on CC levels in the human blood was observed in those conditions. However, this necessitates the evaluation of several health conditions by physicians whenconsidering CC as a renal biomarker. Two main limitations of our study must be pointed out; (i) the small number of recruited samples; thus, the non-parametric statistics were considered. (ii) all the analyzed samples were from the same population and the same region, so our findings might not apply to other populations of different regions. However, our data were comparable with several previously published studies.

Finally, based on the depicted advantages of CC in this work and elsewhere, it is to be hoped that the near future will be more focused on CC as a principal biomarker of renal failure rather than Cr and also of other diseases investigated in this work. Furthermore, we hope that the

studies/investigations will continue; hence, helping people to survive and having a better life.

CLINICAL PERSPECTIVES

1. Different research groups have emphasized the validity of CC as a promising renal failure biomarker, in contrast to Cr, CC doesn't suffer from blind range (a major drawback of Cr), accordingly, CC is highly recommended as a first choice for evaluating renal functions.
2. CC is an ideal biomarker of renal failure. However, several non-renal diseases (investigated in this study and previously) have a noticeable impact on its levels. Therefore, physicians must keep an eye on those diseases should CC is considered as a renal biomarker.

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APPENDIX: SUPPLEMENTARY DATA

Supplemental Table A-1 (a, b, c): CC Levels in Healthy Subjects

Table A-1 (a) CC levels in healthy subjects: non-smoker males ≥ 50 years old

Entry	Gender	Age	CC mg/L
1	Male	57	1.12
2	Male	57	1.71
Min		57.0	1.12
Max		57.0	1.71
Average		57.3	1.41

2 samples, (p = 0.400), non-significant relationship, retain null hypothesis

Table A-1 (b) CC levels in healthy subjects: non-smoker males < 50 years old

Entry	Gender	Age	CC mg/L
1	Male	37	0.88
2	Male	33	0.48
3	Male	19	1.37
4	Male	20	1.19
5	Male	23	0.96
6	Male	8	1.42
7	Male	16	1.13
8	Male	43	1.08
Min		**8.0**	**0.48**
Max		**43.0**	**1.87**
Average		**26.7**	**1.11**

8 samples, (p = 0.400), non-significant relationship, retain null hypothesis

Table A-1 (c) Age in male statistical data[a]

Mann-Whitney U	4.000
Wilcoxon W	40.000
Z	-1.044
Asymp. Sig. (2-tailed)	.296
Exact Sig. [2*(1-tailed Sig.)]	.400[b]

a. Grouping Variable: Age ≥ 50 b. Not corrected for ties.

Supplemental Table A-2 (a, b, c): CC Levels in Healthy Subjects

Table A-2 (a) CC levels in healthy subjects: non-smoker females ≥ 50 years old

Entry	Gender	Age	CC mg/L
1	Female	58	1.18
Min		**58.0**	**1.18**
Max		**58.0**	**1.18**
Average		**58.0**	**1.18**

1 sample, (p = 0.667), non-significant relationship, retain null hypothesis

Table A-2 (b) CC levels in healthy subjects: non-smoker females < 50 years old

Entry	Gender	Age	CC mg/L
1	Female	12	0.76
2	Female	42	0.72
Min		**12.0**	**0.72**
Max		**42.0**	**0.76**
Average		**27.0**	**0.74**

2 samples, (p = 0.667), non-significant relationship, retain null hypothesis

Table A-2 (c) Age in female statistical data[a]

Mann-Whitney U	.000
Wilcoxon W	3.000
Z	-1.225
Asymp. Sig. (2-tailed)	.221
Exact Sig. [2*(1-tailed Sig.)]	.667[b]

Grouping Variable: Females age ≥ 50 b. Not corrected for ties.

Supplemental Table A-3 (a, b, c, d): CC Levels in Healthy Subjects

Table A-3 (a) CC levels in healthy subjects: non-smoker males and females ≥ 50 years old

Entry	Age	Gender	CC mg/L
1	57	Male	1.12
2	57	Male	1.71
3	58	Male	1.18
Min	**57.0**		**1.12**
Max	**58.0**		**1.71**
Average	**41.4**		**1.15**

3 samples, (p = 1.000), non-significant relationship, retain null hypothesis

Table A-3 (b) non-smoker males and females ≥ 50 years old statistical data[a]

Mann-Whitney U	1.000
Wilcoxon W	2.000
Z	.000
Asymp. Sig. (2-tailed)	1.000
Exact Sig. [2*(1-tailed Sig.)]	1.000[b]

a. Grouping Variable: male and female over 50 non-smokers b. Not corrected for ties.

Table A-3 (c) CC levels in healthy subjects: non-smoker males and females < 50 years old

Entry	Age	Gender	CC mg/L
1	37	Male	0.88
2	33	Male	0.48
3	12	Female	0.76
4	42	Female	0.72
5	19	Male	1.37
6	20	Male	1.19
7	23	Male	0.96
8	8	Male	1.42
9	16	Male	1.13
10	43	Male	1.08
Min	**8.0**		**0.48**
Max	**43.0**		**1.42**
Average	**25.3**		**1.00**

10 samples, (p = 0.178), non-significant relationship, retain null hypothesis

Table A-3 (d) non-smoker males and females < 50 years old statistical data[a]

Mann-Whitney U	2.000
Wilcoxon W	5.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.178[b]

a. Grouping Variable: Group

b. Not corrected for ties.

Supplemental Table A-4 (a, b, c): CC Levels in Healthy Subjects

Table A-4 (a) CC levels in healthy subjects: smoker males < 50 years old

Entry	Gender	Age	CC mg/L
1	Male	41	0.73
2	Male	35	1.77
3	Male	34	1.87
Min		**34**	**0.73**
Max		**41**	**1.87**
Average		**36.7**	**1.46**

3 samples, (p = 0.244), non-significant relationship, retain null hypothesis

Table A-4 (b) CC levels in healthy subjects: non-smoker males < 50 years old

Entry	Gender	Age	CC mg/L
1	Male	37	0.88
2	Male	33	0.48
3	Male	19	1.37
4	Male	20	1.19
5	Male	23	0.96
6	Male	8	1.42
7	Male	16	1.13
8	Male	43	1.08
Min		**8**	**0.48**
Max		**43**	**1.42**
Average		**24.9**	**1.07**

8 samples, (p = 0.244), non-significant relationship, retain null hypothesis

Table A-4 (c) Smoking habit statistics data[a]

Mann-Whitney U	11.000
Wilcoxon W	116.000
Z	-1.260
Asymp. Sig. (2-tailed)	.208
Exact Sig. [2*(1-tailed Sig.)]	.244[b]

a. Grouping Variable: Smoker males < 50 b. Not corrected for ties.

Supplemental Table A-5 (a, b, c, d, e)

Table A-5 (a) CC levels in kidney failure patients and eGFR results

Entry	Gender	Age	Creatinine mg/dL	CC mg/L	eGFRCC	eGFRCr	eGFRCr - CC	Stage
1	Male	25	2.89	4.39	12.56	28.88	17.63	Stage 4
2	Male	84	2.25	4.41	9.86	25.80	15.18	Stage 4
3	Male	28	6.55	5.71	8.74	10.51	8.80	Stage 5
4	Male	22	4.80	5.97	8.44	15.98	10.59	Stage 5
5	Female	50	2.59	5.81	0.28	20.83	11.26	Stage 5
6	Male	31	2.66	3.34	17.58	30.62	21.80	Stage 4
7	Male	31	2.66	2.00	34.71	30.62	31.38	Stage 3
8	Male	32	3.30	8.48	5.09	23.38	9.83	Stage 5
9	Female	77	1.95	6.01	0.04	24.36	11.40	Stage 5
10	Female	77	1.95	3.81	0.07	24.36	15.76	Stage 4
11	Female	49	3.65	4.88	0.38	13.84	10.42	Stage 5
12	Female	23	2.70	3.23	4.12	23.92	19.06	Stage 4
13	Female	51	3.16	3.40	0.54	16.29	14.57	Stage 5

Table A-5 (Continued)

Entry	Gender	Age	Creatinine mg/dL	CC mg/L	eGFRCC	eGFRCr	eGFRCr - CC	Stage
14	Female	74	1.96	2.72	0.14	24.71	20.25	Stage 4
15	Female	56	2.53	2.93	0.46	20.51	18.00	Stage 4
16	Male	85	4.21	2.46	21.28	12.03	15.70	Stage 4
17	Male	45	6.51	2.74	21.64	9.39	13.66	Stage 5
18	Male	68	1.92	2.61	21.05	35.10	26.30	Stage 4
19	Male	49	5.78	2.55	23.42	10.56	15.14	Stage 4
20	Male	24	5.90	2.72	23.78	12.27	16.20	Stage 4
21	Male	44	6.83	3.37	16.55	8.94	11.54	Stage 5
22	Female	76	2.70	2.93	0.11	16.48	15.67	Stage 4
23	Female	13	4.56	5.23	4.40	13.64	10.40	Stage 5
24	Female	55	1.83	4.43	0.28	30.57	16.38	Stage 4
25	Female	47	3.44	5.16	0.41	15.11	10.49	Stage 5
26	Male	52	1.50	2.64	22.07	52.58	32.65	Stage 3
27	Female	51	1.46	3.39	0.54	41.41	23.17	Stage 4
Min	13		1.46	2.00	0.04	8.94	8.80	
Max	85		6.83	8.48	34.71	52.58	32.65	
Average	48.9		3.42	3.97	9.58	21.95	16.42	

27 samples[31], ($p < 0.001$), eGFRCC-eGFRCr-CC ($p = 0.008$), eGFRCr-eGFRCr-CC ($p = 0.046$), eGFRCC-eGFRCr ($p < 0.001$), all the mentioned results showed highly significant relationships, reject null hypothesis, refer to Table 2 for eGFR equations.

Table A-5 (b) CC levels in kidney failure patients statistical data[a]

Mann-Whitney U	.000
Wilcoxon W	153.000
Z	-5.532
Asymp. Sig. (2-tailed)	.00000003

a. Grouping Variable: CC mg/L

Table A-5 (c) eGFRCC – eGFRCr-CC equations statistical data[a]

Mann-Whitney U	211.000
Wilcoxon W	589.000
Z	-2.656
Asymp. Sig. (2-tailed)	.008

a. Grouping Variable: eGFRCr-CC

Table A-5 (d) eGFRCr – eGFRCr-CC equations statistical data[a]

Mann-Whitney U	249.000
Wilcoxon W	627.000
Z	-1.998
Asymp. Sig. (2-tailed)	.046

a. Grouping Variable: eGFRCr-CC

Table A-5 (e) eGFRCC – eGFRCr equations statistical data[a]

Mann-Whitney U	142.000
Wilcoxon W	520.000
Z	-3.849
Asymp. Sig. (2-tailed)	.0001

a. Grouping Variable: eGFRCr

Supplemental Table A-6 (a, b)

Table A-6 (a) CC levels in diabetic patients

Entry	**Gender**	**Age**	**Cr mg/dL**	**CC mg/L**
1	Female	21	0.49	2.82
2	Female	58	0.79	2.58
3	Female	53	0.84	1.83
Min		**21**	**0.49**	**1.83**
Max		**58**	**0.84**	**2.82**
Average		**44.0**	**0.71**	**2.41**

3 samples, (p = 0.004), highly significant relationship, reject null hypothesis

Table A-6 (b) Diabetic patients statistical data[a]

Mann-Whitney U	1.000
Wilcoxon W	154.000
Z	-2.593
Asymp. Sig. (2-tailed)	.010
Exact Sig. [2*(1-tailed Sig.)]	.004[b]

a. Grouping Variable: Diabetes

b. Not corrected for ties.

Supplemental Table A-7 (a, b)

Table A-7 (a) CC levels in hyperthyroidism patients

Entry	**Gender**	**Age**	**CC mg/L**
1	Female	53	1.83
2	Female	33	6.98
3	Female	38	5.91
4	Female	28	1.85
Min		**28**	**1.83**
Max		**53**	**6.98**
Average		**38.0**	**4.14**

4 samples, (p = 0.001), highly significant relationship, reject null hypothesis

Table A-7 (b) Hyperthyroidism statistical data[a]

Mann-Whitney U	2.000
Wilcoxon W	155.000
Z	-2.866
Asymp. Sig. (2-tailed)	.004
Exact Sig. [2*(1-tailed Sig.)]	.001[b]

a. Grouping Variable: Hyperthyroidism b. Not corrected for ties.

Supplemental Table A-8 (a, b)

Table A-8 (a) CC levels in hypothyroidism patients

Entry	Gender	Age	CC mg/L
1	Male	38	1.42
2	Female	61	*Below limit of detection (BLD)
3	Male	55	*Below limit of detection (BLD)
4	Female	43	*Below limit of detection (BLD)
5	Female	32	#0.40 Below Limit of quantification (BLQ)
Min		**32**	**0.40**
Max		**61**	**1.42**
Average		**43.7**	**0.91**

*Limit of detection: 0.375 mg/L of CC, # limit of quantification: 0.75 mg/L of CC[31], 2 samples only were included in the statistics, ($p = 0.655$), non-significant relationship, retain null hypothesis

Table A-8 (b) Hypothyroidism statistical data[a]

Mann-Whitney U	13.000
Wilcoxon W	16.000
Z	-.531
Asymp. Sig. (2-tailed)	.595
Exact Sig. [2*(1-tailed Sig.)]	.655[b]

a. Grouping Variable: Hypothyroidism b. Not corrected for ties.

Supplemental Table A-9 (a, b)

Table A-9 (a) CC levels in cardiac dysfunction patients

Entry	Gender	Age	Cr mg/dL	CC mg/L
1	Male	43	1.02	2.38
2	Female	55	0.83	2.45
3	Male	54	1.01	2.62
4	Male	60	0.96	2.57
5	Male	23	0.61	2.79
Min		**23**	**0.83**	**2.38**
Max		**60**	**1.02**	**2.62**
Average		**47.0**	**0.95**	**2.51**

5 samples, ($p < 0.001$), highly significant relationship, reject null hypothesis

Table A-9 (b) Cardiac dysfunction statistical data[a]

Mann-Whitney U	.000
Wilcoxon W	153.000
Z	-3.330
Asymp. Sig. (2-tailed)	.001
Exact Sig. [2*(1-tailed Sig.)]	.00008[b]

a. Grouping Variable: Cardiac dysfunction

b. Not corrected for ties.

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Chapter 6

SPRUE-LIKE ENTEROPATHY

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ABSTRACT

Celiac disease (also termed gluten-sensitive enteropathy or celiac sprue) is a gluten-dependent immune-mediated disorder of the small intestine that occurs in genetically-predisposed persons. Serological studies have estimated that about 1% of screened individuals, possibly more, have celiac disease. The precise precipitating event leading to clinical illness is not known. A number of disorders may have the pathological appearances of celiac disease, such as mucosal injury from oats, other proteins such as soy and a wide array of infections, including protozoans, viral, bacterial and parasitic agents. Some deficiencies including zinc, folic acid and vitamin B12 as well as an immune deficiency syndromes may cause a sprue-like enteropathy. In recent years, medications including pharmacological and biological agents have been recognized ranging from drugs like olmesartan for hypertension to an emerging group of biological agents, particularly checkpoint inhibitors for advanced malignancies.

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Keywords: Celiac disease, Sprue-like enteropathy, Gluten-free diet, Celiac disease mimicry

INTRODUCTION

Celiac disease (gluten-sensitive enteropathy, celiac sprue) is an immune-mediated gluten-dependent disorder that develops in genetically-predisposed persons leading to a complex immune reaction to structural peptides in wheat and other grains, including barley and rye [1, 2]. Although the precise event that precipitates human clinical illness is unknown, diagnosis traditionally has relied on mucosal biopsies from the proximal small intestine followed by a response to gluten-free diet [3, 4]. Classical clinical features that include diarrhea and weight loss usually resolve in the majority with a gluten-free diet. In addition, serological abnormalities including raised antibody levels of IgA tissue transglutaminase fall, often to normal, and pathological changes in the small intestine normalize, initially in the most distal portions of intestinal involvement, and then, later in the proximal duodenum. This improvement may require months, even years, to occur [5]. In some, particularly the elderly defined late in life, extended periods may be needed to show improvement [5].

RECURRENCE AFTER CELIAC DISEASE DIAGNOSIS

In those with well established celiac disease, symptoms, such as diarrhea or weight loss, may recur. Several considerations should result (see Table 1). Usually, recurrence results from failed dietary compliance. Even a so-called “gluten-free” diet may contain trace or measurable amounts of gluten, sufficient to cause recurrent symptoms and persistent inflammatory changes in biopsies [6-8]. Usually, poor compliance is clinically obvious, but sometimes this may be difficult to fully ascertain. In some, gluten consumption may be intentional. Gluten, however, is ubiquitous and may be

found in items like communion wafers and pill capsules so that a strict gluten-free diet avoiding all gluten-free containing products may be difficult. In others, particularly in older children and young adults freed from parental controls, and in the face of increasing peer pressure, gluten consumption may occur. However, if symptoms do recur, other possible causes (besides limited or poor compliance) should be considered.

Table 1. Recurrent symptoms in established celiac disease

Failure of compliance with gluten-free diet
Ubiquitous source of gluten (e. g., pill capsules, communion wafers)
Wrong initial diagnosis (e. g., isolated Crohn's disease of the duodenum)
Associated or second cause (e. g., microscopic, lymphocytic, collagenous colitis)
Superimposed complications (e. g., collagenous sprue, lymphoma)

The original diagnosis of celiac disease may be incorrect. For example, Crohn's disease may occasionally be difficult to differentiate pathologically from untreated celiac disease. In these, diagnosis is especially difficult if Crohn's disease only involves the proximal duodenum, especially early in its pathogenesis [9]. In addition, an entirely new syndrome associated with inflammatory bowel disease has been recognized in patients that have undergone colectomy for isolated, but severe colitis [10, 11]. Indeed with either ulcerative colitis or Crohn's colitis, an extensive post-colectomy enteropathy has also been occasionally recorded [12, 13]. Information on this post-surgical disease is unclear but it is probably more common than currently appreciated and may have an immune-mediated pathogenesis unrelated to the underlying inflammatory bowel disorder.

Recurrent symptoms may also be due to a complication, such as collagenous sprue or lymphoma. In some, it is conceivable that a "treatment-resistant" phenotype of celiac disease is present, or a response may have occurred, but only in the distal small intestine. As noted above, however, response to a gluten-free diet also appears to be temporally-driven, particularly if the initial biopsies are done in males or the elderly [5]. In other words, the response to a gluten-free diet may be sex- and age-dependent [5]. In addition, some may simply be exceedingly sensitive to minute amounts

of dietary gluten. In others, the small bowel simply fails to respond to a gluten-free diet; the disease cannot be defined as gluten-dependent and the term "refractory celiac disease" should not be used. Instead, the label "unclassified sprue" [14] or simply "sprue-like intestinal disease (enteropathy)" appears to be much more accurate. In the past, this entity was thought to represent a heterogeneous group, rather than one disease entity. The histopathological changes are not distinguishable from untreated celiac disease and it has been suggested by others that this is a "wastebasket" diagnosis [14].

In some, abnormal biopsies remain resistant to a gluten-free diet or even worsen. Eventually, however, some of these prove to have a complicating "slow-to-develop" or "slow-to-detect" lymphoma. Clonal expansion of an aberrant, but cryptic, intra-epithelial lymphocyte population has been described (so-called refractory celiac disease, "type II disease"). In these, a specific signature was reported including intracytoplasmic CD3 without surface expression of CD3 and CD8 along with clonally-restricted rearrangement of the T-cell receptor (based on immunohistochemistry or flow cytometry methods) [15, 16].

In rare individuals, an entirely new clinical presentation has been noted. A primarily myopathic process, labeled inclusion body myositis, has been described with sprue-like intestinal disease [17]. In addition to small bowel changes, progressive muscle weakness occurred. Over the course of many years, a gluten-free diet, steroids and multiple nutritional supplements had no impact on either the progressive muscle weakness or the small intestinal mucosa.

SPRUE-LIKE INTESTINAL DISEASE (ENTEROPATHY)

After an initial listing of disorders a half century ago [10], a number of new entities has emerged that may mimic celiac disease. These are shown in Table 2. None of these respond to a gluten-free diet. However, some, particularly infectious agents, may respond to specific treatment, including antibiotics. Others, particularly those related to treatment medications, either

pharmacologic and biologic, may cause sprue-like intestinal disease and often respond completely to simple removal of the offending medication. Although medications may affect the structure and function of either the small or large bowel, sometimes both, some drugs may induce small intestinal changes like untreated celiac disease. In some, underlying celiac disease may have been present, but not initially recognized (e. g., isoretinoin) [18].

Table 2. Disorders with similar biopsy changes to celiac disease

Sprue syndromes
Collagenous sprue
Mesenteric lymph node cavitation syndrome (often with hyposplenism)
Oats-induced villous atrophy and other protein injury (soy, milk)
Infectious causes
Infectious non-bacterial gastroenteritis (?viral agent)
Protozoa (e. g., Giardia lamblia, Isospora belli, Cryptosporidium, Microsporidians)
Bacteria (e. g., Tropheryma whipplei, Mycobacterium), “Tropical sprue”
Parasite (e g., Strongyloides stercoralis)
Stasis syndrome (contaminated small bowel syndrome)
Deficiency syndromes
Nutrients (e. g., zinc, vitamin B12, folic acid) and Kwashiorkor
Immunodeficiency syndromes (congenital, combined, acquired, HIV disease)
Others
Autoimmune enteropathy (“epithelial-antibody positive” enteropathy)
Crohn’s disease of duodenum
Transplant enteropathy (including graft-vs-host disease)
Lymphoproliferative disease (i. e., lymphoma, macroglobulinemia)
Zollinger-Ellison syndrome
Post-gastrectomy or post-colectomy enteropathy
Medication-induced small bowel disease (see Table 3)

Table 3. Medication-induced small bowel disease

Triparanol
Alcohol
Neomycin and Antibiotics
Stathmokinetic Agents (e. g., colchicine, vincristine)
Chemotherapeutic Agents (e. g., methotrexate)
Non-steroidal anti-inflammatory Agents (e. g., sulindac)
Immunosuppressive Agents (e. g., azathioprine, mycophenolate)
Anti-hypertensive Agents (e. g., olmesartan)
Biological Agents (e. g., ipilimumab)

MEDICATION-INDUCED INTESTINAL DISEASE

Pharmacologic Agents

Historically, a drug, triparanol, was used to actually induce a hypothetical animal (i.e., rat) model of celiac disease [1]. This drug was believed to increase the lability of lysosomal membranes leading to intracellular release of enzymes from epithelial cells. Subsequently, other agents were recognized to cause small bowel injury, such as alcohol [20], by a direct focal or diffuse mucosal toxic effect. An indirect effect of chronic alcohol use may also result in mucosal injury due to folic acid deficiency. Folic acid is critical in the process of normal epithelial cell renewal and depletion may result in "megaloblastic" epithelial cells and the appearance of "nuclear-cytoplasmic asynchrony", similar to bone marrow changes associated with folic acid or vitamin B12 deficiencies. Eventually, the rate of renewal is visibly reduced, numbers of mitotic figures are reduced, villi are shortened and the crypts appear hypoplastic (different from the hyperplastic changes of crypt epithelium in untreated celiac disease). Similar effects may occur with other folate depleting agents, including chemotherapeutic agents, such as methotrexate [21]. Antibiotics have also been long recognized to affect the small bowel. Neomycin, for example, has been well studied with documentation of mucosal toxicities with light and

electron microscopic changes and altered absorption of numerous nutrients [22, 23]. Stathmokinetic drugs, like colchicine, may lead to marked mucosal changes including "colchicine spindles" (due to arrested metaphase) along with altered uptake of major nutrients like carbohydrate and fat as well as micronutrients, including vitamin B12 [24]. Vincristine and vinblastine may cause similar effects by disruption or assembly failure of the mitotic spindle [25]. Non-steroidal anti-inflammatory drugs (e. g., sulindac) [26], immunosuppressive agents (e. g., azathioprine, mycophenolate mofetil) have also been implicated in altered small bowel mucosal architectural changes that fail to respond to gluten-free diet treatment [27].

Perhaps, one of the most intriguing pharmacologic agents, recently reported, is olmesartan [28]. This is an angiotensin II receptor antagonist commonly prescribed for the treatment of hypertension. In some, a sprue-like small intestinal disorder has been documented often characterized by diarrhea, weight loss and pathologic features of untreated celiac disease. In some, but not all patients treated with this drug, serological studies for antibodies to tissue transglutaminase were negative and gluten-free diet was not effective in leading to resolution. A number of patients were only recognized after being severely ill, requiring hospital support and medication treatment, including immune suppressants or a biological agent. After cessation of the drug, reversal of clinical and pathological changes have occurred. Similar reversal of biopsy changes have been described with collagenous sprue induced by olmesartan in the absence of any other form of treatment [29].

BIOLOGICAL AGENTS

Another category of medication-related disease is only now becoming more frequently noted. Biological agents classified as immune checkpoint inhibitors, mainly monoclonal antibodies, have been infused in some patients with ongoing and severe inflammatory disorders as well as standard treatment-resistant malignancies, particularly melanoma.

Ipilimumab is a humanized monoclonal antibody to limit the cytotoxic T-lymphocyte antigen 4, a critical negative feedback regulator of the T-cell anti-tumor response. The agent has been used in different malignancies, including metastatic melanoma and metastatic prostate cancer. About 40% develop adverse effects, including an immune-mediated enteritis. If severe, the effects may be fatal. Endoscopic biopsies may also show diffuse enteritis or sprue-like small bowel changes [30]. Treatment has included fluid-replacement, parenteral nutrition, corticosteroids and, in some, infliximab infusions have been used. A sprue-like intestinal disorder with negative serological studies and no apparent response to a gluten-free diet has also been recorded [31]. Other checkpoint inhibitor agents used in treatment of metastatic malignancies, including pembrolizumab and nivolumab have also been noted [32-35].

CONCLUSION

In summary, a diagnosis of celiac disease depends on two criteria. First, a small intestinal biopsy should demonstrate the features of untreated disease, even though these are not specific for the disease; and, second, improvement should be documented after treatment with a gluten-free diet. Antibody testing is a very useful screening measure and offers support for a diagnosis of celiac disease. If the patient fails to unequivocally respond to a gluten-free diet, then a careful exploration for other causes of the biopsy changes should be done. A host of infectious agents should be considered and, if present, specifically treated. Most important, medications may be a critical cause of symptoms and biopsy changes that may permit complete resolution of the celiac-like disorder with removal.

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