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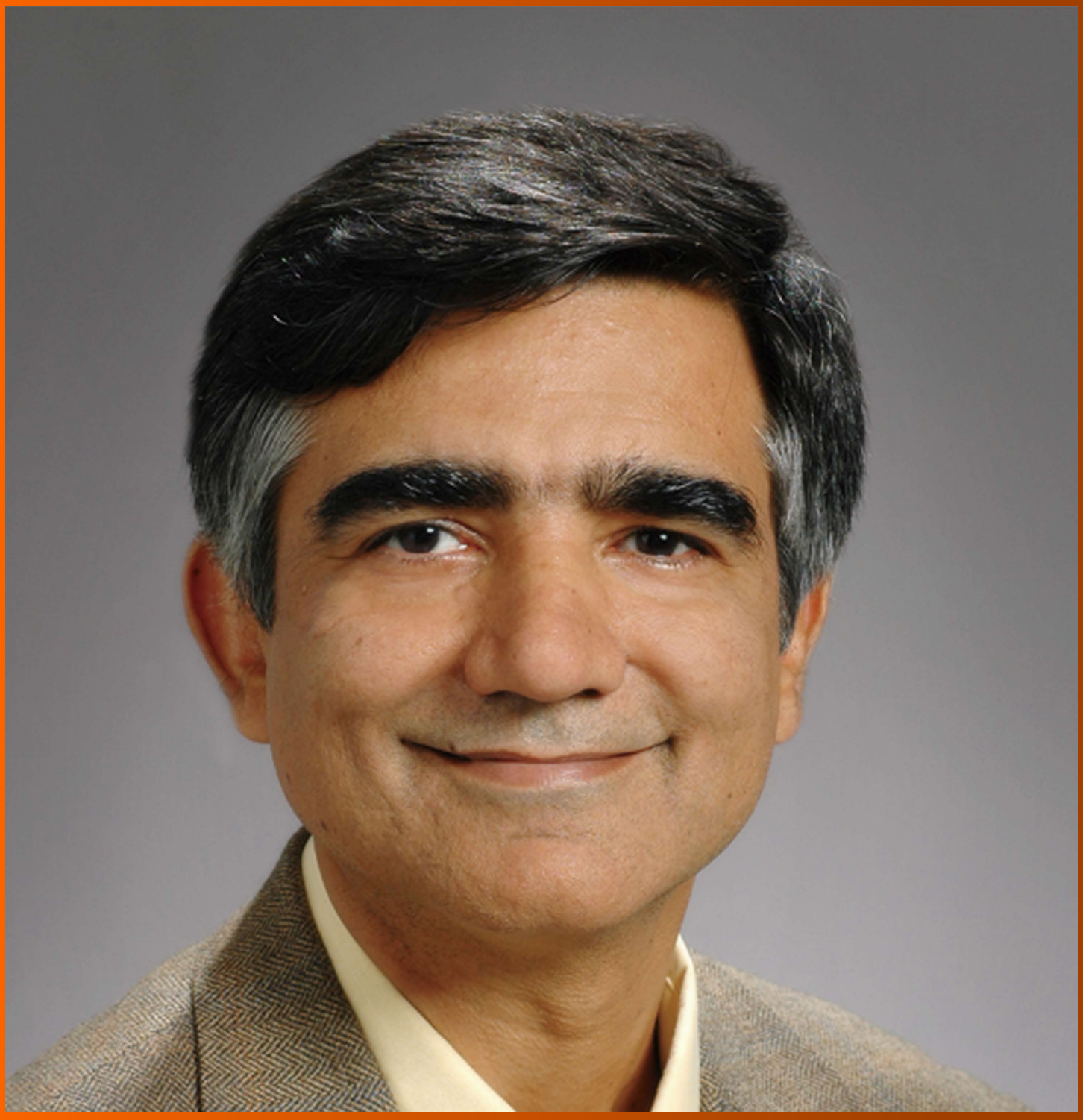

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REVIEW

Circadian dysrhythmia-linked diabetes mellitus: Examining melatonin's roles in prophylaxis and management

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Abstract

Diabetes mellitus is a chronic, life-threatening metabolic disorder that occurs worldwide. Despite an increase in the knowledge of the risk factors that are associated with diabetes mellitus, its worldwide prevalence has continued to rise; thus, necessitating more research into its aetiology. Recent researches are beginning to link a dysregulation of the circadian rhythm to impairment of intermediary metabolism; with evidences that circadian rhythm dysfunction might play an important role in the aetiology, course or prognosis of some cases of diabetes mellitus. These evidences thereby suggest possible relationships between the circadian rhythm regulator melatonin, and diabetes mellitus. In this review, we discuss the roles of the circadian rhythm in the regulation of the metabolism of carbohydrates and other macronutrients; with emphasis on the importance of melatonin and the impacts of its deficiency on carbohydrate homeostasis. Also, the possibility of using melatonin and its analogs for the "prophylaxis" or management of diabetes mellitus is also considered.

Key words: Chronobiology; Dysmetabolism; Insulin; Pancreatic beta cell; Melatonin receptors

Core tip: Diabetes mellitus is a chronic, life-threatening metabolic disorder with a huge disease burden and rising global prevalence that is nearing epidemic proportions. Research has continued to reveal the importance of circadian rhythm and the neurohormone melatonin in the regulation of carbohydrate metabolism. More studies are also revealing the potential roles of melatonin in the pathogenesis, management and mod-

ulation of the course of diabetes mellitus; especially type 2 diabetes mellitus. Presently, an array of potential mechanisms exists for melatonin's roles in diabetes mellitus; however, a complete picture of this is yet to emerge.

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INTRODUCTION

Diabetes mellitus is a chronic, life-threatening metabolic disorder with a huge disease burden and rising global prevalence that is nearing epidemic proportions[1]. According to the World Health Organisation (WHO) diabetes factsheet (which was updated in November 2017), in 2014, 8.5% of adults aged 18 years and older had diabetes mellitus; also, diabetes mellitus accounted directly for about 1.6 million deaths in 2015[1]. There have also been projections that diabetes mellitus will be the seventh leading cause of death by 2030[1,2]. As a disorder, diabetes mellitus is associated with increasing morbidity; accounting for a two- to three-fold increase in the risk of cardiovascular and cerebrovascular disease amongst adults[3]. About 2.6% of global blindness has been attributed to diabetes mellitus[4], and it has also been reported to be a leading cause of chronic kidney disease[5].

Presently, management of type 1 diabetes mellitus (T1DM) relies largely on insulin replacement, while that of T2DM is largely dependent on the use of drugs belonging to classes such as biguanides, sulfonylureas, meglitinides, intestinal brush border glucosidase inhibitors and thiazolidinediones. However, cost is a major limitation to the use of drugs (especially in low-income countries); also, there is the risk of side-effects like weight gain, heart failure and gastrointestinal disturbances[6]. The need to drastically reduce the global prevalence of T2DM necessitates a widening of the search for aetiological factors; and over the last two decades, a growing body of evidence has increasingly suggested the role of the biological clock and multiple clock genes in metabolic homeostasis. Data from epidemiological studies have also shown a correlation between circadian dysregulation (due to urbanisation and/or shift-work) and an increase in the prevalence of cardiovascular disease, cancers, inflammatory disorders, obesity, and diabetes mellitus[7-9]. Along this line, both human and rodent studies have demonstrated such relationships. Scheer *et al*[10] examined the effects of circadian misalignment between the behavioural cycle (feeding/fasting, sleep/wake) and the endogenous circadian rhythm, on metabolic and endocrine predictors of obesity, diabetes, and cardiovascular risk in humans; in their study, they demonstrated that circadian misalignment that occurs acutely (with jet lag) or chronically (with shift-work) was associated with an increased cardiometabolic risk[10]. Genetic polymorphisms involving circadian clock genes and/or circadian locomotor output cycles *kaput* genes have been linked to the development of metabolic syndrome, obesity, T2DM and hypertension[11-14]. *In-vivo* or *in-vitro* rodent studies have also demonstrated a possible link between the disruption of the circadian rhythm[15] or disruption of certain components of the clock genes[16] and the development of hypoinsulinaemia and T2DM.

Evidences demonstrating the importance of chronobiology in intermediary metabolism and the development of diabetes mellitus have also raised questions about the impact that melatonin (a regulator of the circadian rhythm) and its receptors may have on the aetiology, prognosis, prevention and treatment of diabetes mellitus. Certain studies in rodents have reported that melatonin inhibits insulin secretion from beta-cells *via* its interactions with MT1 and/or MT2 receptors on the beta cell-surface[17]; however, in humans, studies using reverse transcription-polymerase chain reaction demonstrated that human islets expressed mRNAs coding for both melatonin (MT1 and MT2) receptors[18]. Results of single-cell microfluorimetry have also suggested that the expression of MT1 receptor mRNA occurred only on alpha-cells and not on beta-cells[19]. An infusion of exogenous melatonin into dissociated human islet cells and perfused human islets, increased intracellular calcium and glucagon secretion respectively[19]. Genetic mapping and genome-wide association studies have also demonstrated strong associations between the gene for melatonin type 2 receptor (*MTNR1B*) which is expressed in the pancreatic beta-cells (amongst other tissues), and an increased risk for T2DM[18,20,21]. Again, there have been reports of impaired glucose tolerance following acute melatonin administration[22]. However, a few other studies have also demonstrated that melatonin receptor signalling in β-cell reduced oxidative stress response, militated against proteotoxicity-induced β-cell apoptosis, and restored glucose-stimulated insulin secretion in normal islets exposed to chronic hyperglycaemia or in type 2 diabetes islets[23].

There is a growing body of knowledge associating alterations in circadian rhythms, circadian genes, melatonin and melatonin receptors with derangement of intermediary metabolism and the development of diabetes mellitus. While the implication of this advance in knowledge for the prevention and therapeutic management of diabetes mellitus is evolving, there are strong indications that β-cell melatonin receptor 2 signalling is relevant for the regulation of β-cell survival and function; and by extension, may also be important in T2DM[23]. In this review, we examine relevant literature for the roles of the circadian rhythm in the physiological regulation of carbohydrates, with emphasis on the importance of melatonin in this capacity. The impacts of melatonin deficiency on carbohydrate homeostasis

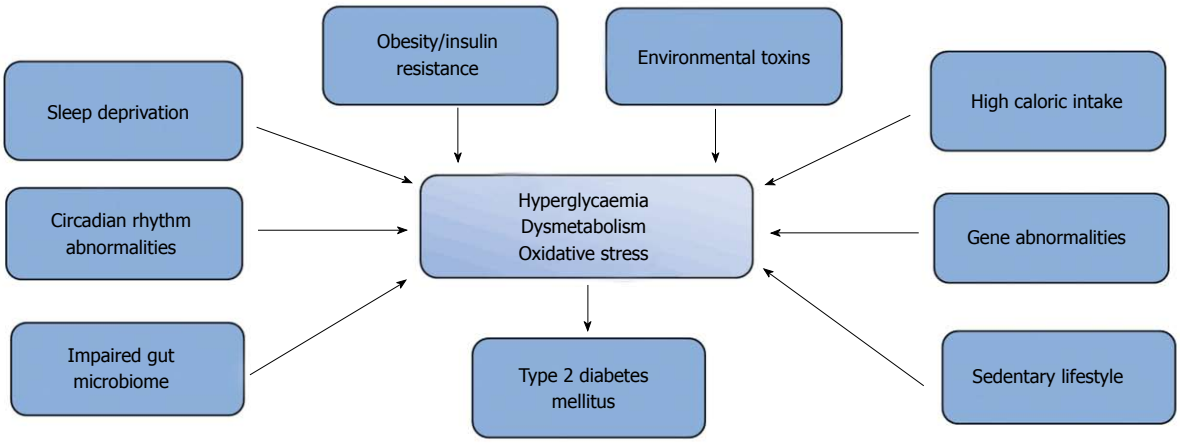

Figure 1 Pathophysiology of type 2 diabetes mellitus.

are also discussed. Finally, the possibility of utilising melatonin and its analogues for the "prophylaxis" and treatment of diabetes mellitus are also considered.

Pathogenesis and molecular basis of T2DM

T2DM, which is characterised by impaired insulin secretion (or sensitivity) and hyperglycaemia, has been reported to account for greater than 90% of the total diabetes mellitus case-load[24,25]. It is a polygenic metabolic disorder that results from the interaction of environmental and genetic factors (Figure 1). These factors include obesity[26,27], sedentary lifestyle, high-calorie diet[28] and sleep deprivation[29]. Recently, reports from epidemiological and animal studies have suggested that increased presence of endocrine disruptors like pesticides, dioxins and bisphenol A in the environment may predispose to insulin resistance, alteration of β-cell function and impairment of glucose homeostasis[30]. While reports from genome-wide association studies have demonstrated strong associations between T2DM and over 100 gene variants that are located at four loci[31]; the peroxisome proliferator-activated receptor gamma gene (*PPARG*), which encodes the nuclear receptor PPAR-γ was the first candidate gene associated with T2DM[32]. Variants of this gene that are expressed in adipose tissue have also been linked to increased transcriptional activity, increased insulin sensitivity and protection against T2DM[22,32]. A number of candidate gene variants including the E23K polymorphisms in *KCNJ11* and P12A in *PPARG* (that have been associated with an increased risk for T2DM)[33] have also been discovered through candidate association studies[34,35]. Studies have also shown that loss-of-function mutations involving *KCNJ11* and *ABCC8* candidate genes are implicated in hyperinsulinemia in infancy[36].

There have been reports that gut microbiota are important in the maintenance of gastrointestinal mucosa permeability, metabolism of dietary polysaccharides (to produce short-chain fatty acids) and the regulation of fat accumulation[37]. These functions make them crucial to the development of obesity and obesity-related diseases[38]. Differences in gut microbiome between lean and obese subjects have also been reported[39]; with suggestions that an important role exists for gut bacteria (and possibly their end-products) in intermediary metabolism. Studies have also demonstrated that alteration in gut microbiota is associated with the development of T2DM and its complications[40]. The importance of gut microbiome to T2DM is affirmed by studies that have shown that transplantation of faecal microbiome from lean donors to subjects with insulin-resistance results in beneficial metabolic changes[41]. Studies in rodents have also demonstrated that modulation of the gut microbiome may also be beneficial in T2DM management[42].

A growing body of evidence suggests an important role for adipose tissue and lipotoxicity in T2DM. Presently, adipose tissue is considered an endocrine organ which influences lipid and glucose metabolism[43,44]. Dysfunctional adipose tissue (characterised by adipocyte hypertrophy, impaired insulin signalling and insulin resistance) results in the release of inflammatory adipokines and large amounts of free fatty acids; causing fat accumulation and lipotoxicity in organs involved in glucose metabolism such as liver, muscle and pancreatic beta cells[45-47]. Reports from a number of human and animal studies have also demonstrated the importance of brown adipose tissue in glucose homeostasis and the regulation of energy expenditure; with the possibility of brown adipose tissue becoming a therapeutic target[48-50].

Another area of extensive research into the pathophysiology of T2DM is the role that oxidative stress plays in the pathogenesis of micro- and macro-vascular diabetic complications[51]. It is believed that oxidative stress (*via* a common mechanism that involves the production of superoxide, and the inactivation of endothelial nitric oxide synthase and prostacyclin synthase) leads to the development of β-cell dysfunction, insulin resistance, impaired glucose tolerance, and T2DM[52,53]. There have also been suggestions of the involvement of this common mechanism in the development of both microvascular and macrovascular complications as-

sociated with T2DM[53,54]. Studies have also shown that T2DM associated increase in oxidative stress occurs as a consequence of hyperglycaemia, hyperinsulinaemia, insulin resistance, and dyslipidaemia[51].

CIRCADIAN RHYTHM

The circadian rhythms can be defined as endogenous rhythms (with behavioural and physiological components) that have a periodicity of about 24 h, and are synchronised through both photic and nonphotic stimuli[55]. These rhythms are known to control important biological processes, including sleep-wake cycle, hormone secretion, body temperature regulation, feeding/energy homeostasis, and cell-cycle regulation[55]. The circadian system is composed of a master clock which is located in the suprachiasmatic nucleus (SCN) of the hypothalamus and a number of peripheral clocks, which together regulate daily variations in many biological processes[56]. The suprachiasmatic nucleus is responsible for generating the circadian rhythms and as such is referred to as the endogenous biological pacemaker[57]. Daily adjustments of the timing of the SCN following exposure to stimuli (*zeitgebers*) which signals time of day helps to achieve synchrony with the earth' s rotation. A loss of the coordination of these rhythms is known to negatively impact body physiology and behaviours[55].

Anatomically, the SCN is a bilateral structure that contains over 20000 neurons and is a central component of the circadian timing system[56]. It receives input pathways for light and other stimuli that are important in the synchronisation of the pacemaker to the environment; output rhythms are in turn regulated by the pacemaker[55]. Direct (retinohypothalamic) and indirect (retinogeniculate) photic information to the SCN comes from the retina[58]. Retinohypothalamic photic information originates from the ganglion cells of the retina (which contain melanopsin, and are regarded as the primary photoreceptors for the circadian system), nonphotic information comes from the raphe nuclei, while other afferents come from the pons, medulla, basal forebrain and posterior hypothalamus[55]. Arising from the SCN, major efferents project to areas such as the hypothalamus (dorsomedial, subparaventricular zone and the paraventricular nucleus), thalamus, preoptic/retrochiasmatic areas, stria terminalis, lateral septum, and intergeniculate nucleus[55]. Gamma-amino butyric acid is the dominant neurotransmitter that is found in the SCN; however, the SCN core contains vasoactive intestinal polypeptide, gastrin-releasing peptide and bombesin-containing neurons, while somatostatin and neurophysin are predominant within the shell[55].

Circadian timing is affected by several *zeitgebers* including light, feeding schedules, activity, and the hormone melatonin; of these, light is considered of utmost importance, and the most potent stimulus[55]. Light also modulates pineal gland melatonin secretion through regulation by the SCN, with peak secretion occurring in the middle of the night[57]. Another important marker of internal time (especially during periods of low ambient light) is the circadian rhythm of pineal melatonin. The timing of the endogenous circadian rhythm can be determined by dim light melatonin onset (DLMO) which is regarded as a stable marker of the circadian phase[57]. Melatonin is also associated with the maintenance of sleep propensity rhythm in humans, and as such, it is considered a modulator of internal sleep[59]. There is also evidence suggesting that exogenous melatonin can induce phase shifts in the circadian clock[59].

The genetic control of the circadian rhythms is determined by a core set of *clock* genes which interact with their own products to form a number of molecular feedback loops, which regulate the circadian rhythm[60]. These genes include three *period* (*Per*) *homolog* genes (*Per1*; *Per2*; *Per3*), two plant cryptochrome gene homologs (*Cry1*and *Cry2*), the circadian locomotor output cycles kaput gene (*Clock*) and the cycle gene (*Bmal1*)[60]. The interactions of these genes and their products form transcription-translation (molecular) feedback loops that generate the circadian rhythm, and also controls the temporal expression of a number of clock-controlled genes[61].

Circadian rhythm dysregulation and intermediary metabolism

The circadian rhythm is a conserved timing system that modulates behavioural and physiological process to 24-h environmental cycles[55,62]. It is generally accepted that the circadian rhythm depends on *zeitgebers* or cues for the daily adjustments of its timing; as such, daily cycles of activity/feeding and the biological/molecular rhythm assist in the maintenance of energy homeostasis, linking the circadian clock to metabolic systems[63]. It is known that the molecular clock is present in all metabolic tissues including the liver, intestine, adipose tissue, heart, and retina[62]. This master clock in the SCN works in synchrony with the peripheral clocks, and together, they regulate cellular and physiological functions[64]. Some of these functions which include metabolism and energy homeostasis occur through organs such as the liver, and other peripheral tissues. A part of this task is achieved by regulating the expression and/or activity of certain key metabolic enzymes and transport systems that are involved in the lipogenic and adipogenic pathways[64,65]. However, this relationship is bidirectional, with the metabolic enzymes and transcription activators also interacting with and affecting the clock mechanism. An understanding of this relationship is crucial to appreciating how abnormalities such as mutations in clock genes can disrupt cellular rhythmicity and metabolic homeostasis. Also, clinical studies that focus on shift workers and obese patients further illuminate the link between the circadian clock and energy metabolism[64,65].

There are strong indications that circadian misa-

lignment (or dysfunction) is an emerging risk factor for metabolic diseases[62]. Studies have shown that variations in diet or dietary intake may influence the circadian rhythm of feeding/activity; and this in turn modulates the biological or molecular clock[63]. A number of studies have also associated circadian rhythm disruption and sleep loss/deprivation with obesity[66,67]. Studies in humans who are on night-time shift work also demonstrated that strong associations exist between alterations in circadian rhythm and metabolic parameters such as increased body mass, increased plasma lipid, and glucose levels[68-70]. Karatsoreos *et al*[71] reported that chronically housing mice in an environment with shortened light/dark cycle resulted in weight gain, alteration of body temperature rhythms, and increased plasma levels of leptin and insulin[71]. Several disorders relating to human psychology and sleep have also been associated with abnormal functioning of the master biological clock. A number of the core hormones that are involved in nutrient metabolism (including insulin, glucagon, adiponectin, corticosterone, leptin and ghrelin) have been shown to undergo circadian oscillation in their levels and activities[72-74]. Studies have also demonstrated that the molecular clock controls mitochondrial posttranslational modification and oxidative metabolism[75]. The molecular clock controls cellular metabolism through its ability to direct the rhythmic synthesis of nicotinamide adenine dinucleotide (NAD^+), which is a metabolic cofactor. NAD^+ subsequently modulates the activity of the protein deacetylase, sirtuin 1 (SIRT1), which controls cellular metabolism *via* a feedback loop[76-78]. These nutrient sensors relay information about the cellular nutrient status to the circadian clock, and modulate the activity of clock genes. For example, while the oxidised forms of sodium dehydrogenase (NAD^+) redox co-factor inhibits the activity of heterodimers of circadian clock genes like *Clock/Bmal1* and *Npas2/Bmal1*; the reduced forms (NADH) increases their activity[79]. Others, like AMP kinase have also been shown to regulate expression of clock genes[80-83]. Studies in which deletions or mutations in the clock genes result in disruption of the cellular rhythm also provide strong evidence of the cross-talk that occurs between the circadian clock and metabolism.

There are also reports suggesting that key proteins may be involved in the regulation of the core clock mechanism and adipose tissue metabolism; thereby linking the circadian rhythms with lipid metabolism[65]. The role of the circadian clock in the regulation of adipose tissue differentiation has been considered[84]. *In-vitro* and *in-vivo* studies have also been used to examine the role of the circadian rhythm in adipocyte physiology. Studies involving cell lines in which clock genes transcription factors like *Bmal1* or *Rev-Erbα* (a nuclear receptor which suppresses *Bmal1* expression) were knocked out reported inhibition of adipocyte differentiation[85-87]; while those involving mutations of clock components like *Per2*[88] or retinoid orphan receptor α[89,90] were associated with an increase in adipogenesis, with these effects mediated by PPARγ[91,92]. Studies in male mice have also demonstrated that the rhythm of expression of the clock genes and adipose PPARγ are decreased by the consumption of high-fat diet[93].

Circadian rhythm and glucose control: Like all other aspects of intermediary metabolism, blood glucose homeostasis is also under circadian regulation; with variations in blood glucose levels occurring with the changes in external synchronisers (activity/feeding and resting/starvation)[94]. During the activity/feeding phase, blood glucose levels are maintained from dietary intake; whereas, during the resting/starvation period, there is a progressive recruitment of glucose from endogenous glucose sources in the liver to maintain blood levels within a relatively narrow margin[94]. The liver also alternates between glycogenolysis and glycogenesis[95,96]. Studies have also shown that daily blood glucose control is also modulated by both the central circadian clock in the SCN as well as by peripheral clocks in the pancreas, liver, muscle and white adipose tissue. This is affirmed by studies in humans, that have observed differences in glycaemic response between meal studies conducted in the morning and those in the evening[97-99]. This alteration in glycaemic control had been attributed to circadian variations in insulin secretion and an increase in hepatic or peripheral insulin resistance[99-101]. Studies using animal models have also shown that insulin secretion follows a rhythmicity that is regulated by peripheral pancreatic β-cell clocks[102]. In humans there have been reports that the set-point for the regulation of the 24-h pulsatile secretion of insulin is higher in obese subjects, T2DM subjects, and their non-diabetic first degree relative[102,103] compared to the general population. Studies using different animal models of circadian clock gene dysfunction (*ClockΔ19, Cry1 and Cry2, Bmal1*) have also reported evidence of hyperglycaemia, increased insulin sensitivity or impaired insulin secretion[16,104,105].

Gut hormones which are very important in modulating gastric emptying and maintaining glucose homeostasis, like the anorexigenic peptides (glucagon, insulin, glucose inhibitory peptide, glucagon-like peptide-1, amylin, peptide YY) and the orexigenic hormone ghrelin have also been shown to fluctuate with activity/feeding and resting/starvation periods. The variations in their activity pattern are also under circadian control and as such may be altered by circadian disruptors, including altered meal times, dietary compositions and constant light exposure[106,107].

There are evidences supporting the existence of a relationship between gut microbiota and the circadian system; and presently, it is known that intestinal microbiome is regulated by circadian rhythms through the intrinsic circadian clocks[108]. This regulation affects host metabolic function through alteration of microbial community structure as well as their metabolic activities. Up to one-fifth of human gut bacteria exhibit diurnal variations in their activities and abundance; and some species, like *Enterobacter aerogenes* had been shown

to be responsive to the circadian fluctuations in the hormone melatonin[108]. Alterations in the balance of this relationship can lead to changes in the activities and relative composition of gut microbiota. Finally, abnormalities in composition and activities of gut microbiota had been linked to insulin resistance and diabetes mellitus through several mechanisms, such as regulation of adiposity/obesity, regulation of the immune system, modulation of inflammatory processes, and extraction of energy from the diet[109].

Circadian rhythm dysfunction, sleep and T2DM

A number of studies have demonstrated that a dysregulation of the internal circadian clock system or discordance with the external environmental cues has deleterious health consequences, with an associated increase in morbidity and mortality in humans[9]. Increasingly, results from epidemiological[110,111] and animal[8,9,15] studies continue to show associations between circadian rhythm dysfunction (that occur due to sleep loss, shift work or nocturnal lifestyle) and the development of T2DM[8,9,15] (Figure 2). An *in-vitro* study using rat pancreatic islets revealed that exposure of the islets to continuous light was associated with a disruption of the circadian clock function and reduction in glucose-stimulated insulin secretion, due to a decrease in insulin secretory pulse mass[112]. Also, there have been reports that a disruption of circadian rhythm could induce abnormal insulin release in people at risk of developing T2DM. Gale *et al*[15] examined the metabolic and physiological changes associated with T2DM following circadian rhythm dysfunction in wild-type, Sprague Dawley and diabetes-prone human islet amyloid polypeptide transgenic rats that were exposed to prolonged episodes of normal light (or experimental disruption in the light-dark cycle), and reported that circadian rhythm disruption accelerated the development of diabetes in diabetes-prone rats, but not in wild-type rats[15]; an effect that has been attributed to pancreatic β-cell loss and dysfunction[15]. Marcheva *et al*[16] reported that disruption of the clock gene components (*Clock* and *Bmal1*) was associated with delays in the phase of oscillation of islet genes that were involved in islet cell growth, glucose metabolism and insulin signalling; resulting in impaired glucose tolerance, reduction in insulin secretion, and alterations in the size and proliferation of pancreatic islets[16]. They also demonstrated that conditional ablation of the pancreatic clock resulted in the development of diabetes mellitus *via* alteration in β-cell function[16]. Also, there have been suggestions and experimental evidence to show that the mammalian islet clock was responsible for regulating the expression of genes that are involved in sensing glucose levels, insulin secretion, as well as islet cell growth and development[16,113].

While we gain new insights into the pathophysiology of T2DM, and continue to understand the roles played by the circadian rhythm[15,16,113]; there is ample scientific evidence to show that a disruption of circadian rhythms alters not only the body weight and adiposity, but it also affects glucose metabolism and glycaemic control. While the magnitude of these effects (as it relates to the development and progression of T2DM) continues to be studied, it is also important to continue to investigate their precise mechanisms, and to determine the relevance of this new knowledge to the therapy and prevention of T2DM.

There appears to be strong relationships between certain sleep parameters and the risk of development of diabetes mellitus. Along this line, numerous evidences from both epidemiological and laboratory studies have continued to reveal and support the fact that poor sleep is strongly associated with the development of glucose-intolerance, insulin resistance, and ultimately T2DM[114].

In a community-based study of adults of both sexes in Xuzhou, China; it was found that after adjustment for a large number of possible aetiological factors, poor sleep-quality and short (⩽ 6 h) sleep duration were significantly associated with increased prevalence of diabetes mellitus, when compared with the group of people with good quality of sleep and longer (6-8 h) overnight sleep duration[115]. Again, poor sleep has been known to be associated poor glycaemic control in T2DM patients. In a Japanese study involving 3249 patients with T2DM; an assessment of sleep, using the Pittsburgh Sleep Quality Index (PSQI) showed that (independent of potential confounders) poor subjective sleep quality was associated with less-than-optimal glycaemic control[116].

MELATONIN

Melatonin is a tryptophan-derived indoleamine which is primarily secreted by the pineal gland, with contributions from a number of other tissues including the retina, bone marrow, gastrointestinal tract, skin, ovary and placenta[117,118]. The extra-pineal contribution to melatonin production is small when compared to secretion from the pineal gland; with suggestions that it is only triggered by some specific impulses[119]. Melatonin secretion is regulated by the central circadian clock, as well as by seasonal variations in length of daylight. Production is acutely suppressed by exposure to light, with increased secretion occurring at night in both nocturnal and diurnal species. Plasma concentrations of endogenous melatonin also vary considerably with age[120-122]. Melatonin is a multifunctional molecule that is capable of intracrine, paracrine or autocrine signalling[117]. It can cross all physiological barriers and exert widespread regulatory effects on numerous body tissues. Melatonin is important in the regulation of biologic rhythms[123]; and numerous studies in humans and rodents have reported melatonin's widespread influence on varied biological and behavioural processes[124-126]. Melatonin plays important roles in neurogenesis, neuroprotection and the maintenance of oxidant/antioxidant balance[127-129]. A few studies have also reported its role in diabetes control[18].

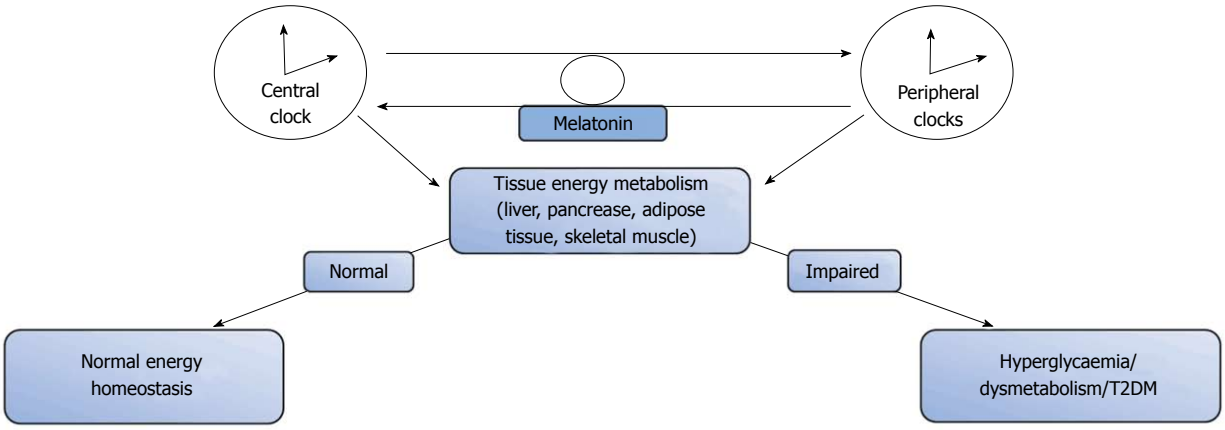

Figure 2 The role of circadian rhythm in the development of type 2 diabetes. T2DM: Type 2 diabetes mellitus.

Melatonin's role in intermediary metabolism

There is increasing scientific evidence to suggest that a derangement of melatonin rhythmicity may have adverse health implications, especially as it relates to its importance in modulating a variety of metabolic functions, as well as its role as a regulator of epigenesis[130]. Studies have demonstrated the presence of high concentrations of extra-pineal melatonin in the gastrointestinal tract (GIT) of a number of mammals[131]. There had also been reports suggesting that extra-pineal melatonin from the GIT contributes significantly to circulating blood melatonin levels (mostly during the day)[131]; although there are evidences to suggest that some of the melatonin in the GIT may be pineal in origin. However; there are reports suggesting that the release of GIT melatonin may be related to the periodicity of food intake rather than being photoperiodic, as occurs with melatonin from the pineal gland[131]. These evidences are stimulating interest in investigating the possible relationship that may exist between melatonin in the GIT and metabolism; especially, since a number of studies in vertebrates had demonstrated exogenous melatonin's ability to modulate appetite, energy metabolism, anorexigenic hormone/peptide concentration, and body weight[132-134]. Earlier studies evaluating melatonin's relationship with the GIT and intermediary metabolism reported alterations in the overall food consumption in mice following administration of exogenous melatonin; while a few other studies also demonstrated an increase in tissue and blood melatonin levels with food intake and prolonged food deprivation[131,135]. Studies in zebrafish (*Danio rerio*) had also demonstrated that melatonin administration induced a decrease in food intake, it also modulated the stimulation of satiety and anorexigenic signals in the liver and intestine[136]. However, a number of studies have suggested that melatonin's roles in appetite modulation may arise from different mechanisms; with suggestions that its anorexigenic effects could be as a result of its ability to delay gastric emptying[137,138] or *via* its stimulatory activity on fat mobilisation[139,140]. A number of other studies in fish have also reported that melatonin's ability to reduce food consumption may be related to circadian rhythm stimulation (*i.e.*, its ability to promote sleep), and not necessarily due to a direct effect of the hormone[141].

Melatonin, melatonin receptors, glucose metabolism and T2DM

A number of studies have provided evidence that melatonin influences glucose metabolism. In healthy subjects, glucose homeostasis is controlled within a narrow margin *via* a complex pathway of regulatory mechanisms that involves multiple organs and tissues (Figure 3). Therefore, a disruption of normal glucose balance usually results from a sustained reduction in both pancreatic beta-cell function and insulin secretion[142,143]. In rodents, melatonin has been shown to regulate blood glucose concentration through its ability to bind directly to melatonin receptors on hepatocytes[144] and regulate the uptake of glucose in adipocytes, by modulating the expression of the glucose uptake transporter[145]. Abnormalities of the nocturnal melatonin profile have also been described in diabetic patients, especially in those suffering from diabetic neuropathy[146]. Low melatonin secretion is also independently associated with a higher risk of developing T2DM; an association that further establishes the roles of melatonin in glucose metabolism and insulin sensitivity[147]. Post mortem studies have also indicated an association between diabetes mellitus and decreased melatonin secretion[148]; while some *in-vivo* and *in-vitro* studies have demonstrated melatonin's ability to inhibit the secretion of insulin by pancreatic beta-cells[149]. Presently, a growing body of evidence suggests a relationship between disturbances in melatonin production and impairment of insulin, glucose and lipid metabolism[134,150]; and that of antioxidant capacity[130,151,152]. Results from both *in-vivo* and *in-vitro* studies have shown that in patients with metabolic syndrome, night-time melatonin level is related to night-time insulin concentrations[153]. There have also been reports of lower elevations in night-time melatonin levels in diabetic subjects; raising interests in the link between

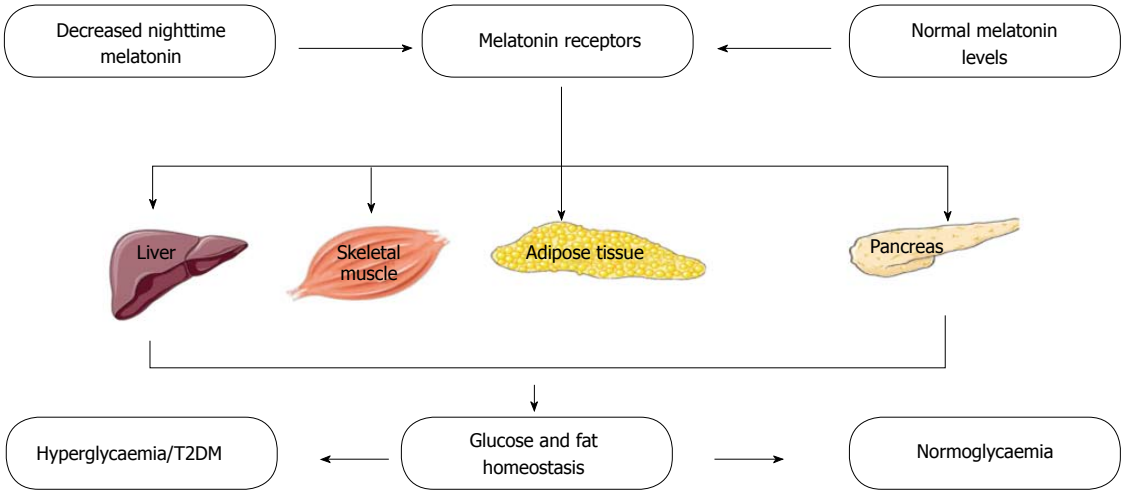

Figure 3 Melatonin, melatonin receptors, glucose and fat homeostasis and type 2 diabetes mellitus. T2DM: Type 2 diabetes mellitus.

melatonin and hyperglycaemia/diabetes mellitus[154]. Also, melatonin has been reported to stimulate the secretion of glucagon, another hormone that is important in glucose metabolism[155].

Melatonin receptors (MT1 and MT2) have been observed to be present in rodent[156-158] and human[18,19,159] pancreatic islets. The expression of these receptors also varies with the circadian rhythm and feeding status[160]. In humans, several genetic studies have associated MT2 receptor polymorphisms with an increased risk of developing T2DM[150]. Associations between single nucleotide polymorphisms that are situated close to (or within) the gene that encodes MT2 (MTNR1B), and an increased risk of developing T2DM[18,161,162], diminished B-cell function[163,164] and impaired glycaemic control[165-167] have all been reported in cohorts of different regions and ethnicities. Studies have also demonstrated an increase in the expression of MT1 and MT2 receptors in the pancrease of diabetic rats and in subjects with T2DM[168].

Melatonin's potential roles in prophylaxis or treatment: Experimental and clinical data continue to suggest that both endogenous as well as exogenously-administered melatonin play crucial roles in the improvement of diabetes control. In a rat model of diabetes mellitus, long-term administration of melatonin (1.1 mg/d for 30 wk) attenuated the development of hypertriglyceridaemia, hyperinsulinaemia and hyperleptinaemia[169]. In a study among community-dwelling diabetics, the effect of administration of 2 mg of prolonged-release melatonin (at 9-11 pm for 3 wk) on glucose and lipid metabolism was investigated[148]. This initial administration was followed by an extended period of five months of open-label, prolonged-release melatonin administration to evaluate the effects of prolonged-release melatonin on glycosylated haemoglobin (HbA1c) levels[148]. The results established the safety of prolonged-release melatonin with regards to parameters such as glucose, lipid metabolism, and other routine biochemical indices; also, there were no adverse interactions with routinely-used anti-diabetic drugs, or insulin release[148]. In an earlier study involving twenty-two postmenopausal non-diabetic women, the results suggested that glucose tolerance and insulin sensitivity are reduced following a single oral administration of melatonin 1 mg[170]. However, in diabetic women, use of prolonged-release melatonin (in the short term or long term) did not impair insulin action or glucose tolerance; on the contrary, there was improved glycaemic control upon long-term use[148]. A few other studies have demonstrated that melatonin plus zinc acetate alone, or in combination with metformin improved both fasting and postprandial glycaemic control in T2DM patients[171].

Presently, research continues to unravel the multifaceted effects of melatonin on intermediary metabolism, especially that of glucose; with direct evidences of melatonin's effects on insulin secretion, pancreatic beta cell activity, hepatic glucose metabolism and insulin sensitivity[172]. Apart from these, melatonin also combats cellular/tissue oxidative stress and inflammation. Therefore, by the modulation of several intracellular signalling pathways and tissue targets, melatonin is emerging to occupy a central role in the understanding of the aetiology and management of diabetes mellitus[173].

Melatonin receptors (MT1 and MT2) have been shown to be present on human pancreatic islets, and the effects of melatonin on insulin secretion are mediated through these receptors[17]. Melatonin is able to affect insulin secretion in two ways, decreasing it by inhibiting cAMP and cGMP pathways, and increasing it by activating the phospholipase C/Inositol triphosphate pathway, which mobilises calcium ions from organelles, consequently increasing insulin secretion. Melatonin also induces production of insulin growth factor and promotes insulin receptor tyrosine phosphorylation; while its supplementation attenuates glucose intolerance and insulin resistance[17].

The use of melatonin in the pharmacotherapy of

diabetes mellitus may confer additional benefits over what is obtainable with conventional drugs alone. This is due to its ability to affect several pathways that may be involved in the pathogenesis or progression of the disease. In an experimental model designed to express obese T2DM phenotype, rats with concomitant circadian disruption and diet-induced obesity were treated daily with oral melatonin, metformin, or a combination of the two for 12 wk[174]. It was observed that melatonin alone improved circadian activity/rhythms, attenuated induction of beta-cell failure, and enhanced glucose tolerance. Use of metformin alone only enhanced insulin sensitivity and glucose tolerance. However, combining melatonin with metformin attenuated progression of metabolic dysfunction by improving adiposity, circadian activity, insulin sensitivity, and islet cell failure[174]. The results suggest that attenuation or arrest of circadian dysfunction may be crucial to managing metabolic dysfunction and altering the course of the disease in T2DM. In mice that were given high-fat diet (HFD), oral melatonin at 100 mg/kg per day (for 10 wk) led to a significant reduction in body weight-gain (compared to the HFD controls) and it also reduced hepatic steatosis. Also, there was improved insulin sensitivity and glucose tolerance, with down- regulation of fetuin-A (a hepatokine that is associated with insulin resistance and T2DM) and endoplasmic reticulum stress markers in the liver and serum[175].

One of the ways by which melatonin may be beneficial in the management of T2DM and metabolic syndrome is through its ability to reduce adiposity by modulation of the gut microbiota. In mice that were fed high-fat diet, melatonin treatment significantly reversed gut microbiota dysbiosis, increasing the ratio of the bacteria that are known to be associated with a healthy mucosa while also improving markers of adiposity and inflammation[176].

Some studies have also assessed the impact of melatonin supplementation on the development of microvascular and macrovascular complications of diabetes mellitus and concluded that melatonin has beneficial effects in repairing cardiac injury due to diabetes mellitus[177]. Zhou *et al*[176] reported that inhibition of the splenic tyrosine kinase (which is activated by hyperglycaemia and contributes significantly to the development of diabetic cardiomyopathy) by melatonin supplementation reversed diabetes-related loss of myocardial function, decreased cardiac fibrosis and preserved the viability of cardiac myocytes[176].

There have been studies that had reported the influence of melatonin on mitochondrial bioenergetics due to its ability to regulate mitochondrial fission/fusion[178,179] and regulate mitophagy/autophagy[180]. In view of the above, Ding *et al*[181] examined the possible effects of melatonin supplementation on the development of myocardial contractile dysfunction (which has been linked to an increase in mitochondrial fission in subjects with diabetes mellitus), and reported that melatonin attenuated diabetes-induced myocardial dysfunction by decreasing the expression of dynamin-related protein 1, leading to the prevention of mitochondrial fission[181]. Melatonin administration also prevented mitochondrial fragmentation, decreased oxidative stress, and reduced apoptosis of the cardiomyocyte in streptozotocin-induced diabetic mice; however, these were not replicated in the protein deacetylase sirtuin 1 (SIRT1)$^{-/-}$ diabetic mice[181]. Thus, suggesting that melatonin's cardioprotective effects were exerted through its effects on SIRTI[181].

Melatonin's antioxidant or oxidative stress-reduction effect is one of the benefits that have increased interests in its possible use in the management of diabetes mellitus and its complications. Studies in rodents have demonstrated that intraperitoneal administration of melatonin (3 mg/kg per day for 4 wk) reduced lipid peroxidation marker (malonyldialdehyde) and increased glutathione levels in the bone tissue of diabetic rats subjected to acute swimming exercise[182]. Mehrzadi *et al*[183] also examined the effects of melatonin supplementation on the development of diabetes-related retinal injury in rats. Their results showed that while induction of diabetes increased oxidative stress and inflammation, treatment with melatonin for a period of seven weeks attenuated the development of retinal injury; largely through reduction of oxidative stress and inflammation[183]. Studies in human subjects have also demonstrated that melatonin's cardioprotective effects can be attributed to its ability to reduce oxidative stress and improve cardiometabolic risk[184]. In a randomised, double-blind, placebo-controlled trial, two groups of subjects were administered either melatonin (10 mg) or placebo, once daily for 12 wk[184]. Results of this study showed that (compared to subjects that were administered placebo) melatonin supplementation (in addition to its beneficial effects on glycaemic control, reduction of insulin resistance and improvement of insulin sensitivity) was associated with an increase in the plasma concentration of glutathione, nitric oxide, high density lipoprotein; and a decrease in the levels of malondialdehyde and serum C-reactive protein[184].

A few studies in rodents have also explored the possible use of melatonin as an adjunct to insulin therapy. Oliveira *et al*[185] reported that 8 weeks of administration of melatonin in drinking water at 0.2 mg/kg body weight (either alone or in combination with insulin (NHP, 1.5 U/100 g/d) improved glycaemic control, increased insulin sensitivity and reduced the expression of hypothalamic genes that are related to reproductive function[185].

CONCLUSION

Research has continued to reveal the importance of circadian rhythm regulation, and the neurohormone melatonin in the regulation of carbohydrate metabolism. More studies are also revealing the potential roles of melatonin in the pathogenesis, management and modulation of the course of diabetes mellitus, especially T2DM; and as shown by these studies, an array of possible mechanisms exists for melatonin's effects.

However, a complete picture of the role(s) of melatonin in the management of DM is yet to emerge. Also, we

are yet to get to the point where melatonin and melatonin receptor agonists may be prescribed as adjuncts or alternatives to already-existing orthodox medications. Finally, we are just beginning to understand how melatonin may be used to prevent or delay the occurrence of diabetes mellitus.

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REVIEW

Role of bisphosphonates in the management of acute Charcot foot

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Abstract

Diabetes mellitus is the most common cause of Charcot neuropathy affecting foot and ankle. Acute Charcot foot (CF) presents with a red and swollen foot in contrast to the painless deformed one of chronic CF. Enhanced osteoclastogenesis plays a central role in the pathogenesis of acute CF. Many studies have shown elevated levels of bone turnover markers in patients with acute CF confirming it. These findings have led clinicians to use anti-resorptive agents [bisphosphonates (BP), calcitonin, and denosumab] along with immobilization and offloading in acute CF patients. The maximum evidence among all anti-resorptive agents is available for BPs, although its quality is low. Pamidronate has been shown to reduce the markers of activity of CF like raised skin temperature, pain, edema, and bone turnover markers in the majority of studies. Intravenous BPs are known to cause acute phase reactions leading to flu-like illness following their first infusion, which can be ameliorated by oral acetaminophen. Alendronate is the only oral BP used in these patients. It needs to be taken on an empty stomach with a full glass of water to avoid esophagitis. The side-effects and contraindications to BPs should be kept in mind while treating acute CF patients with them.

Key words: Charcot foot; Diabetes mellitus; Charcot neuroarthropathy; Bisphosphonates; Pamidronate

Core tip: Bisphosphonate is an attractive treatment option for acute Charcot foot. This is based on the fact that increased osteoclastic activity plays a central role in the pathogenesis of acute Charcot foot. Among bisphosphonates, the maximum evidence in the literature is available for pamidronate. It has been shown to reduce the markers of Charcot foot activity, like raised skin temperature, pain, and edema. However, the quality of evidence is low. They should be used along

with immobilization and offloading. The side effects of bisphosphonates and their contra-indications for use should be kept in mind while treating these patients with them.

Durgia H, Sahoo J, Kamalanathan S, Palui R, Sridharan K, Raj H. Role of bisphosphonates in the management of acute Charcot foot. *World J Diabetes* 2018; 9(7): 115-126 Available from: URL: http://www.wjgnet.com/1948-9358/full/v9/i7/115.htm DOI: http://dx.doi.org/10.4239/wjd.v9.i7.115

INTRODUCTION

Charcot foot (CF), also quoted as Charcot neuroarthropathy (CN), derives its name from Jean-Martin Charcot, who in 1868 first described neuroarthropathic changes in patients with tabes dorsalis[1]. It was not until 68 years later, in 1936, that William Riley Jordan first established the association between diabetes mellitus (DM) and painless neuropathic arthropathy of ankle[2]. It is a rare and devastating condition leading to the destruction of bone and joints and culminating in fractures, dislocations, deformities, and amputation of the foot in neglected cases. Virtually any condition that causes neuropathy can lead to CF, such as DM, syphilis, leprosy, spinal cord injury, meningomyelocele, syringomyelia, chronic alcoholism, and a host of other conditions like psoriasis, sarcoidosis, rheumatoid arthritis, human immunodeficiency virus, and Parkinson's disease. Currently, the world is witnessing an exponential rise in the prevalence of DM and its complications. According to the World Health Organization 2016 report, around 422 million people are living with DM[3]. This has made DM the most common cause of CN affecting foot and ankle. The incidence of CF in diabetic patients ranges between 0.1% and 7.5%[4].

Today, the pathophysiology of CF is still a bone of contention even after one and half centuries since its first description. Conventional theories for it include the neurovascular theory postulated by Charcot himself and the neurotraumatic theory proposed by Volkmann and Virchow[5]. Peripheral sensorimotor neuropathy along with autonomic dysfunction is the essential factor for the development of CN. Usually a trivial trauma in the insensate foot kicks off the inflammatory cascade. Not all neuropathic patients, however, develop CF.

Recent advancements in the understanding of the pathophysiology of CF has shed light on factors like inflammatory cytokines and their interaction with receptor activator of nuclear factor kappa-B (RANK), its ligand (RANKL), and osteoprotegerin (OPG)[6]. Long-standing hyperglycemia, with its complications ranging from neuropathy to formation of advanced glycation end products (AGEs)[7] and protein kinase C (PKC) activation[8], is the major culprit. Calcitonin gene-related peptide (CGRP)[9], Wnt/beta-catenin pathway[10], and OPG gene polymorphisms[11] are new players in the field. Interaction between RANKL, nuclear factor kappa-B (NF-κB), and pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), Interleukin-1β (IL-1β), and interleukin-6 (IL-6) lead to localized osteolysis that destroys bone structure[12]. Moreover, DM patients have lower 1,25(OH)$_2$D$_3$ levels, leading to poor mineralization of bone[13]. Lower calcium levels can stimulate parathyroid hormone, thus contributing to bone resorption and osteopenia[14].

Studies have shown increased levels of osteoclastic resorption markers, such as serum carboxyterminal telopeptide of type 1 collagen (1CTP), in patients with CF[15]. Immobilization and avoidance of physical stress by complete offloading with the help of total contact cast (TCC) is the mainstay in the management of CF[16]. However, because of increased osteoclastic activity, the bone destruction continues unabated. Lower limb osteopenia seen in patients with CN along with increased bone resorptive markers make anti-resorptive agents like bisphosphonates (BPs), calcitonin, and denosumab reasonable treatment options, at least for adjuvant purposes. BPs are pyrophosphate (PP) analogs that have been in medical use for around half a century. First generation BPs like etidronate and clodronate have non-nitrogen containing side chains, whereas second and third generation ones like pamidronate, alendronate, ibandronate, risedronate, and zoledronate have nitrogen containing side chains. Nitrogen containing BPs are much more potent than the first generation ones and work by inhibiting farnesyl PP (FPP) synthase in the mevalonate pathway, which is crucial for function and survival of osteoclasts[17]. In this study, we reviewed the available literature on the use of BPs in patients with acute CF.

CLINICAL PRESENTATION

Clinically, CF can present either in acute or chronic stage, and its features vary according to the stage of presentation. A high index of suspicion is required to diagnose CF in its early stage. Acute CF presents with a red and swollen foot, which is warmer than the contralateral normal foot. Patients may have mild to moderate pain or discomfort at this stage, which is much less when compared to those with a similar degree of inflammation without neuropathy[16,18]. Skin temperature difference of $\geqslant 4^{\circ}$ Fahrenheit (or 2° Celsius) between affected and the normal foot indicates active CF[19]. This can be measured using an infrared thermometer at the maximum point of deformity on the affected foot and at the same point on the normal foot. It is also helpful in monitoring the course of CF. Peripheral pedal pulses are typically bounding because of underlying autonomic neuropathy. Clinical presentation at this stage mimics those of deep vein thrombosis, acute gout, and cellulitis, and the diagnostic dilemma is compounded by the inability of radiographs to detect and differentiate these abnormalities. Magnetic resonance imaging can be helpful at an early stage of disease[20]. If treatment is not

provided at this stage, it leads to further destruction of bone resulting in irreversible damage. Chronic CF is characterized by resolution of inflammation and establishment of residual deformity. Rocker bottom deformity is the classic abnormality that arises due to collapse of plantar arch in mid foot[21]. This results in abnormal high pressure areas on the weight bearing sites of the plantar surface, making it prone to ulceration[22].

PATHOGENESIS OF ACUTE CHARCOT FOOT

Conventional theories

Two age-old theories pertaining to the pathogenesis of CF that are still pertinent include neurovascular theory and neurotraumatic theory. Neurovascular theory[1] suggests that damage to trophic or vasomotor nerves secondary to the underlying condition results in failure of vasoregulation, causing opening of arteriovenous shunts. This leads to the increased supply of blood to the bone, resulting in greater flux of monocytes and osteoclasts and culminating in bone resorption. Other factors, like peripheral vascular disease, are expected to co-exist with diabetic neuropathy. This leads to decreased blood flow to lower limbs, which can act as a protective factor against CF[23]. This probably explains why CF affects only a fraction of DM patients with neuropathy.

On the other hand, Volkmann and Virchow in their neurotraumatic theory suggested that trauma to the insensate foot leads to CN[5]. Repeated microtrauma in a patient with sensory neuropathy leads to bone destruction and deformity. Though both feet of susceptible patients have the propensity to develop CF, only the one exposed to recurrent trauma develops CF. This provides some ground for the pathogenesis of unilateral CF in the background of generalized neuropathy. However, it has been found to be bilateral in 9% to 39% of cases[24]. With the passage of time, we have now come to know that CF results from the combination of these processes. Autonomic neuropathy weakens the bone because of increased blood supply, whereas sensory neuropathy causes loss of protective sensation leading to unperceived recurrent trauma to the abnormal bone. Muscle weakness due to motor neuropathy adds fuel to the fire, leading to joint instability and abnormal plantar pressures[25]. These progress later to bone fracture and dislocation in foot and ankle.

Other factors that play a role in the pathogenesis of CF are: (1) inflammatory cytokines; (2) AGEs; and (3) neuropeptides and inorganic molecules. These mediators finally stimulate osteoclastogenesis, leading to bone loss *via* RANKL/OPG pathway (Figure 1).

Role of inflammatory cytokines

In addition to his neurovascular theory, Charcot recognized inflammation as one of the contributors to CN. Christensen *et al*[26] in their study showed that hyperemia during an acute attack of CF was most likely secondary to the inflammation rather than sympathetic neuropathy. Thus, it is unabated inflammation in the background of neuropathy that results in the imbalance between osteoclasts and osteoblasts leading to bone resorption. This pro-inflammatory state can be triggered by repeated microtrauma. Hyperglycemia in DM can lead to increased PKC activity and formation of AGEs along with decreased phosphatidylinositol 3 kinase activity[8]. This, in turn, results in an excessive production of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6. This storm of pro-inflammatory cytokines disturbs osteoclast-osteoblast homeostasis. Baumhauer *et al*[12] histologically examined 20 tissue biopsy specimens obtained from patients with CF. Immunohistochemical study of each of these biopsies showed positivity for IL-1, IL-6, and TNF-α. This was conclusive of stimulation of osteoclastic progenitor cells, leading to osteoclastogenesis by the cytokines present in the background during acute and reparative stages of CF. But inflammatory cytokines alone do not directly account for the increased osteoclastogenesis. Jeffcoate *et al*[27] suggested that inflammatory cytokines lead to increased osteoclastogenesis *via* increased expression of NF-κB. This results in bone destruction, which again potentiates the inflammatory response thus culminating in a vicious cycle[27]. Increased cytokines lead to increased activity of RANKL that in turn activates RANK, which is expressed on osteoclast precursors. Increased RANK stimulates intracellular pathways, leading to increased formation of NF-κB. NF-κB stimulates differentiation of osteoclast premature cells to mature osteoclasts, culminating in increased osteoclastic activity. Simultaneously, NF-κB up regulates expression of OPG, a decoy receptor for RANKL, which effectively antagonizes its activity[28]. Ndip *et al*[29] in their study showed that patients with CN have elevated RANKL/OPG ratio and illustrated that abnormal RANKL/OPG signaling plays a crucial role in increased osteoclastic bone resorption. Another bone regulating pathway involving Wnt/β-catenin has been speculated to have some role in bone remodeling in patients with CF[10]. To date, the RANKL/OPG pathway defect remains the most accepted theory.

Role of hyperglycemia

Glycation of collagen occurs normally with aging[30]. Hyperglycemia accelerates this process of non-enzymatic glycation, leading to the formation of Amadori products. These products combine with amino groups on other protein molecules, ending up in formation of the AGEs, which are known to play a major role in various complications of diabetes[31]. AGEs cause irreversible posttranslational modification of proteins, thus rendering them defective. Binding of AGEs to their receptor (RAGE) stimulates nicotinamide adenine dinucleotide phosphate oxidase[32], resulting in the production of reactive oxygen

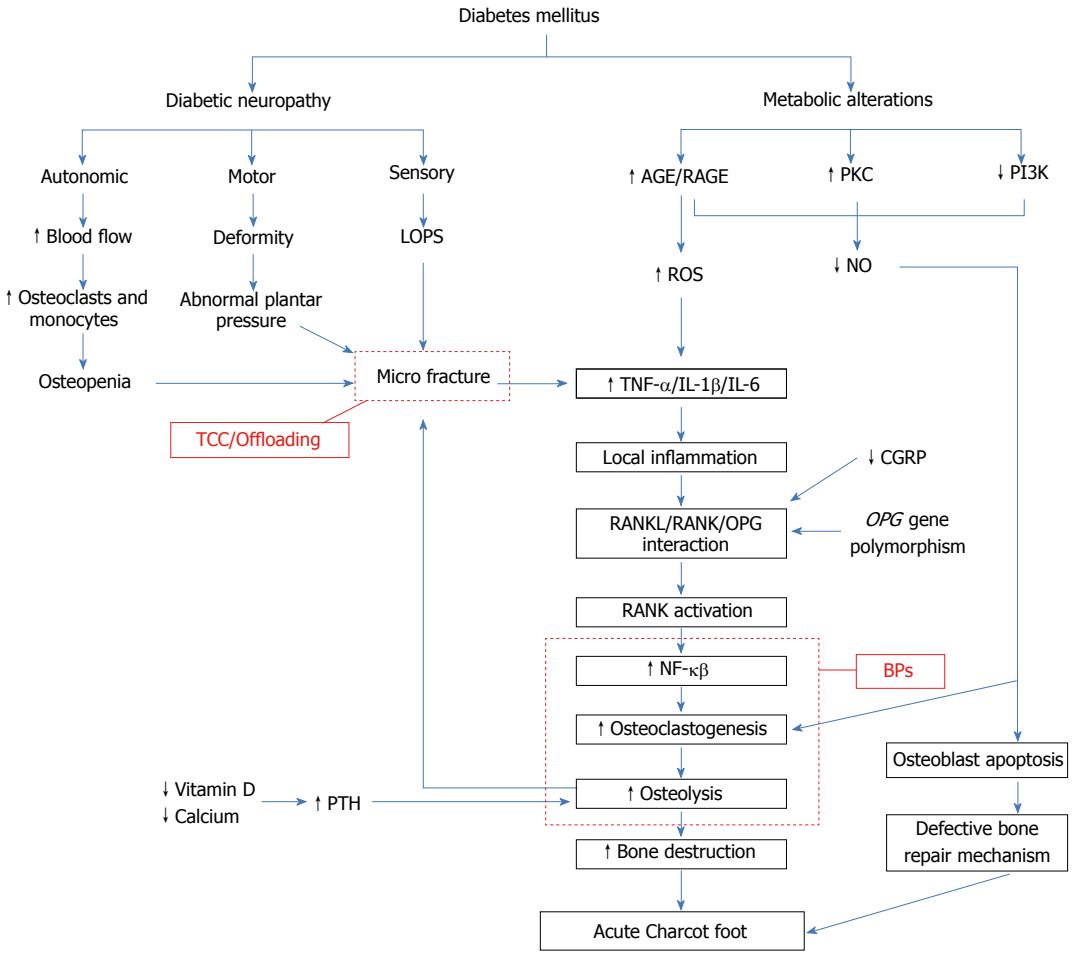

Figure 1 Pathogenesis leading to acute Charcot foot. AGE: Advanced glycation end products; RAGE: Receptor of AGE; PKC: Protein kinase C; PI3K: Phosphatidylinositol 3 kinase; LOPS: Loss of pain sensation; ROS: Reactive oxygen species; NO: Nitric oxide; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; TCC: Total contact cast; CGRP: Calcitonin gene related peptide; NF-κβ: Nuclear factor κβ; RANKL: Receptor activator of NF-κβ ligand; OPG: Osteoprotegerin; BP: Bisphosphonates; PTH: Parathyroid hormone.

species and subsequently increased expression of NF-κB[33]. Katayama *et al*[34] elucidated the effects of AGE-modified collagen on differentiation and function of the osteoblastic cell *in vitro* and suggested that the same changes may lead to osteopenia in diabetic patients. AGEs prevent differentiation of human mesenchymal stem cells[35]. They stimulate apoptosis of osteoblasts through mitogen activated protein kinase and cytosolic apoptotic pathways that are independent of NF-κB activation[36]. AGEs also cause endothelial dysfunction by extinguishing nitric oxide (NO) activity[37]. Soluble RAGE (sRAGE) is a C-terminal splice variant of RAGE and has been shown to be cytoprotective against AGE[38]. Witzke and colleagues in their cross-sectional study concluded that patients with CN had lower levels of sRAGE compared to healthy controls and diabetic patients without CN[7]. They also demonstrated a positive correlation between sRAGE levels and calcaneal bone stiffness suggesting that sRAGE has a protective effect against bone resorption and loss of sRAGE defense may be one of the factors leading to CN. Thus, AGEs lead to increased osteoclastogenesis *via* the RANKL/NF-κB pathway and decreased bone formation by their action on osteoblasts through multiple pathways.

Role of neuropeptides and inorganic molecules

Research has shown that feedback mechanisms are abnormal in patients with DM, leading to increased expression of RANKL. One such mechanism involves CGRP secreted from the healthy neurons. It antagonizes RANKL expression by increasing the release of anti-inflammatory cytokines like IL-10[14]. This leads to inhibition of osteoclastogenesis. The release of CGRP is reduced in peripheral and autonomic neuropathy, leading to continuous unchecked RANKL activity[39]. NO is an inorganic molecule that plays a role in CN. AGEs, along with increased PKC expression and decreased phosphatidylinositol 3 kinase activity, results in decreased production of NO. Studies have shown that decreased NO levels can stimulate osteoclastogenesis, thereby leading to bone resorption[40]. Endothelial NO synthase (eNOS) also regulates osteoblast proliferation and function[41,42]. eNOS knockout animals have been shown to develop osteoporosis secondary to defective

bone formation[42]. Both these molecules were studied by La Fontaine and colleagues in their study[9]. They performed immunohistological analysis of bone specimens from three groups of patients with DM: group 1 included healthy patients without neuropathy, group 2 included those with neuropathy, and group 3 included those with CN stage Ⅱ or Ⅲ. They observed decreased levels of CGRP in patients in groups 2 and 3 when compared to group 1. They also found a statistically significant difference in the levels of eNOS, with highest levels in healthy DM patients without neuropathy (group 1) and lowest levels in DM patients with CN (group 3).

ROLE OF ANTIRESORPTIVE THERAPY IN ACUTE CHARCOT FOOT

Increased osteoclastic activity is the essence of pathogenesis leading to CF. Many studies have shown elevated levels of bone turnover markers (BTMs) in patients with acute CF pointing towards this fact.

Gough *et al*[15] compared BTMs between four groups of patients: acute CF, chronic CF, diabetic controls, and non-diabetic controls. They concluded that levels of serum 1CTP were significantly elevated in patients with acute CF as compared to the other three groups (P < 0.0001). Jostel *et al*[43] in their review mentioned similar results with urinary cross linked N-telopeptides of type 1 collagen, pointing towards accelerated collagen breakdown in these patients. However, levels of serum procollagen type Ⅰ carboxy-terminal propeptide did not show intergroup differences.

These findings have forced researchers to use antiresorptive agents along with traditional immobilization in acute CF patients. To date, agents like BPs have been used in multiple studies (discussed later). BPs are the principal agents in the pharmacological armamentarium against diseases, where the osteoblast-osteoclast imbalance is the underlying pathology. They are analogues of inorganic PP binding to hydroxyapatite crystals, which have extremely high affinity for bone mineral. They get deposited in mineralized bone matrix and are released at the time of bone resorption. This high affinity for bone mineral and resultant uptake by activated osteoclasts at the time of resorption ensures its toxic accumulation only in osteoclasts. First generation non-nitrogen containing BPs are metabolized to cytotoxic adenosine triphosphate analogues by osteoclasts. Intracellular deposition of these toxic non-hydrolyzable analogues causes apoptosis of osteoclasts[44]. Unlike their predecessors, second and third generation BPs like alendronate, pamidronate, ibandronate, risedronate, and zoledronate have nitrogen side chain bound to the central carbon, which magnifies their potency manifolds. The mechanism by which nitrogen containing BPs impacts osteoclast activity and survival differs from that of the first generation BPs. After getting internalized, they inhibit FPP synthase, a key enzyme in the mevalonate pathway, which is responsible for production of cholesterol and isoprenoid lipids[45]. As a result, isoprenylation of guanosine triphosphate binding proteins like Ras, Rho, and Rac is inhibited[46]. These signaling proteins are important for the regulation of cell survival, proliferation, and cytoskeletal organization. Of particular importance among these is inhibition of protein prenylation and Ras signaling within osteoclasts, resulting in defective intracellular vesicle transport[47]. Thus, osteoclasts fail to form ruffled borders, which are necessary for resorption of bone. In addition to this, FPP synthase inhibition leads to an increase in isopentenyl diphosphonate, which is further metabolized to triphosphoric acid 1-adenosin-5′-yl ester 3-[3-methylbut-3-enyl] ester, also known as ApppI. Intracellular accumulation of this ATP analogue leads to apoptosis of osteoclasts (Figure 2). Potency of BPs is decided based on the inhibition of FPP synthase activity. In this respect, zoledronate is the most potent BP followed by risedronate, ibandronate, alendronate, and pamidronate with decreasing potency[17]. Moreover, in animal studies, BPs have shown to possess antinociceptive effects that can contribute to pain relief in patients with acute CF[48,49].

Other anti-resorptive agents like calcitonin and denosumab have been successfully used in past. Calcitonin is a polypeptide secreted from parafollicular C cells of the thyroid. It inhibits bone resorption by its direct action on the osteoclast calcitonin receptor[50]. Its quick action leads to loss of ruffled border of osteoclasts and decreased number of osteoclasts. It inhibits cytoplasmic motility and generates pseudopodial retraction in osteoclasts[51]. It prevents the production and release of tartrate-resistant acid phosphatase by osteoclasts[52]. It has also been shown that calcitonin may inhibit apoptosis of osteocytes and osteoblasts[53]. To evaluate the effects of calcitonin on disease activity, Bem *et al*[54] conducted a randomized controlled trial on the effectiveness of intranasal salmon calcitonin 200 IU daily in 32 diabetic patients with acute CF. One group received intra-nasal salmon calcitonin 200 IU daily and calcium supplementation, while the other got only calcium supplements. All patients were offloaded using removable devices. Skin temperature and BTMs (measured monthly for first 3 mo and then at 6 mo) were used for monitoring the course of treatment. Nine patients with renal insufficiency, *i.e.,* serum creatinine > 120 μmol/L, were also included. Skin temperature reduced significantly at 3 mo without much inter-group difference. Significant reduction was noted in levels of 1CTP in the treatment group at 3 mo as compared to control group (P < 0.01). A similar trend was observed for bone-specific alkaline phosphatase (ALP) at 3 mo (P < 0.05), but the intergroup difference disappeared at 6 mo. The authors concluded that intranasal calcitonin not only reduces bone resorption and prevents progression of acute CF but also can be effective in patients with renal insufficiency. Calcitonin also has analgesic action mediated through central as well as peripheral mechanisms[55,56].

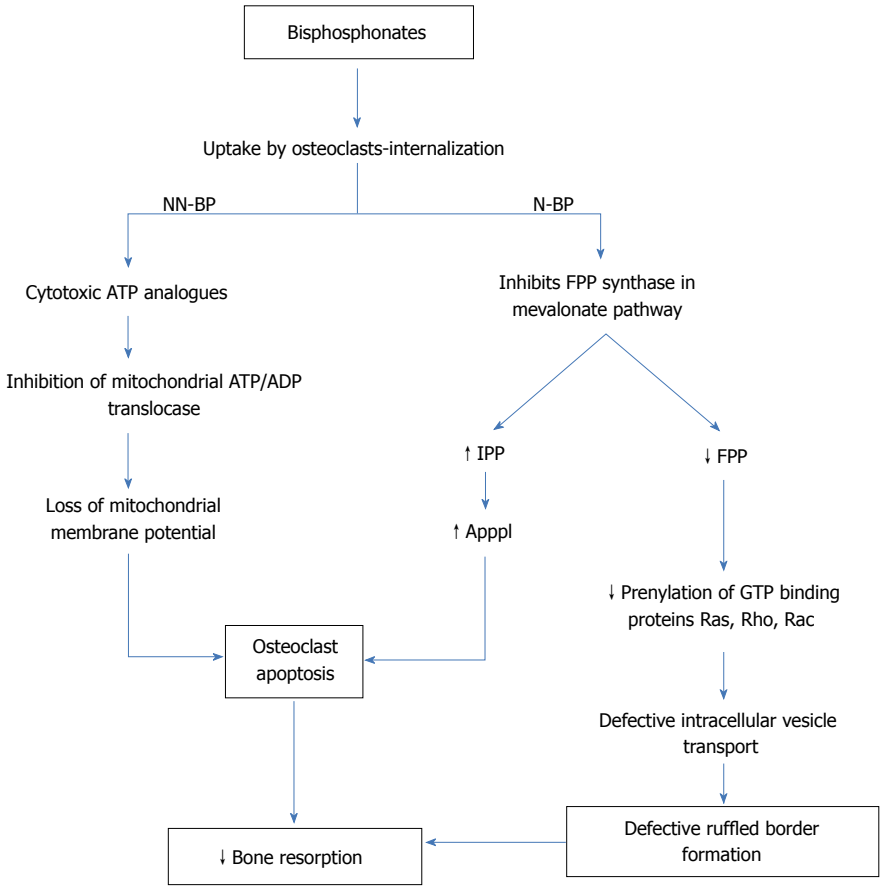

Figure 2 Molecular mechanisms of bisphosphonates. NN-BP: Non-nitrogen containing bisphosphonates; N-BP: Nitrogen containing bisphosphonates; FPP: Farnesyl pyrophosphate; IPP: Isopentenyl diphosphonate; Apppl: Triphosphoric acid 1-adenosin-5'-yl ester 3-[3-methylbut-3-enyl]ester.

As RANKL activation plays a major role in the pathogenesis of acute CF, its inhibition can be an attractive treatment option. Denosumab is a fully human monoclonal antibody that targets RANKL. It prevents interaction between RANKL and its receptor RANK. This leads to inhibition of RANKL, which in turn prevents differentiation of osteoclast precursors to mature multinucleated osteoclasts. The basic difference between BPs and denosumab is that the former act after getting internalized, while the latter works in an extracellular environment[57]. It has been shown to reduce osteoporosis-related fracture[58]. Taking cues from this work, Busch-Westbroek *et al*[59] performed an observational study to evaluate effects of denosumab in patients with acute CF. Patients seen between 2012 and 2014 were included as controls, and those from 2014 to 2016 were subjected to single subcutaneous injection of 60 mg denosumab. All the patients from 2012 to 2016 were immobilized using TCC and were supplemented with calcium and vitamin D. Fracture resolution time, as judged on radiographs and time to clinical cessation based on usage of TCC, were compared between the two groups. Both parameters were significantly shorter in the group receiving denosumab ($P < 0.01$). TCC was used until resolution of edema, and skin temperature difference between both feet decreased to less than 2℃ in this study.

EVIDENCE OF BISPHOSPHONATE USE IN ACUTE CHARCOT FOOT

Case reports and case series

In 1994, Selby *et al*[60] first reported use of intravenous (IV) pamidronate in six diabetic patients with acute CF. Patients were treated with infusion of 30 mg of pamidronate followed by five infusions of 60 mg every 2 wk. Skin temperature, as a marker of disease activity, was monitored by an infrared thermometer. All patients reported marked improvement in their mobility and reduction in pain and swelling. Skin temperature difference between the affected and normal foot reduced from 3.4 ± 0.7 ℃ to 1.0 ± 0.5 ℃ ($P = 0.05$). Serum ALP, which was used as BTM, was also significantly reduced (by 25 ± 3%, $P < 0.001$).

In 1999, Young MJ[61] reported two diabetic patients with CF who were treated with IV infusion of 30 mg of pamidronate followed by two infusions of 60 mg every 2 wk along with immobilization measures. In both patients, skin temperature difference normalized (*i.e.*, < 2 ℃), edema and pain subsided after 3 mo, and there was no deformity in the lower limbs.

In 2002, Yu *et al*[62] reported a case of recurrent CF in a 55-year-old diabetic patient. He was treated with serial Jones compression bandages followed by non-

weight bearing brace with a removable pneumatic walker along with three IV infusions of pamidronate each 2 wk apart. The patient improved clinically, swelling disappeared, and he resumed full weight bearing with an ankle-foot orthosis.

In 2002, Pakarinen *et al*[63] retrospectively studied 36 CF in 32 diabetic patients. Eighteen cases received IV pamidronate 30 to 60 mg once a wk for 6 wk. They did not find any difference in casting time between patients who received pamidronate and those who did not (11 wk *vs* 13 wk). There was no information regarding criteria used for removal of casts or the indication for BP use in a particular patient. This along with non-uniformity in the timing of cast usage make it difficult to analyze the results of this study.

In 2007, Moreno *et al*[64] prospectively analyzed the efficacy of pamidronate over 12 mo in four diabetic patients with acute CF. Treatment protocol comprised of three IV infusions of pamidronate each 2 mo apart. The dose used was 60 mg in patients with weight < 70 kilograms and 90 mg in patients with weight > 70 kilograms. Clinical examination, radiographs, and urine BTMs were done before and 12 mo after treatment in all patients. All patients exhibited significant clinical improvement. Urinary BTMs showed a statistically significant reduction. All patients had radiological improvement.

In 2008, Naqvi *et al*[65] reported three diabetic patients with acute CF. First patient, a 54-year-old female was treated with three IV infusions of 90 mg of pamidronate every 2 mo. After the first infusion, the patient had marked clinical improvement in swelling, pain, erythema, and warmth. Following the second infusion, she was able to bear weight on her foot, and after the last infusion she was ambulant without the walker. The second patient was a 49-year-old African-American female, who was treated with single IV infusion of 60 mg pamidronate along with walking cast and physiotherapy. At 6 and 9 mo follow-ups, signs of inflammation disappeared. Although the natural arch of the foot was lost, she was able to walk with a boot. The third patient was an 82-year-old white woman, who was treated with a single 90 mg of pamidronate infusion. This led to significant clinical improvement, and she was able to walk with the boot at 4 wk of follow-up. One year later, she had no symptoms and was able to walk normally.

In 2002, Rajbhandari *et al*[23] in their review revealed their anecdotal experience in patients with acute CF. They noted significant symptom relief in these patients with two IV infusions of 90 mg pamidronate.

Observational studies

In 2004, Anderson *et al*[66] retrospectively evaluated 33 patients of acute CF who were diagnosed between October 1997 and January 2001. These patients were divided in two study groups - group 1 comprising of 18 patients who received IV pamidronate (60 to 90 mg) and group 2 comprising of 15 patients who did not receive any BPs. Both groups received standard immobilization measures. Finally, after excluding five patients each from groups 1 and 2 due to either lack of consent for treatment or bilateral CF or association with some other bone disease or infection, 13 patients from group 1 and 10 patients from group 2 were analyzed. In group 1 patients, limb temperature decreased by 2.8 °F at 48 h and 7.4 °F at 2 wk, whereas group 2 showed no reduction in temperature at 48 h and a reduction of 2.3 °F at 2 wk. The same trend was seen in serum ALP, which plummeted by 53% at 2 wk in group 1 and showed a meager reduction of 9% in group 2. Thus, this study demonstrated a statistically significant reduction in skin temperature and serum ALP in patients treated with pamidronate.

In the largest web-based observational study published in 2012, Game *et al*[67] surveyed 288 diabetic patients with acute CF from 76 centers across the United Kingdom and Ireland. At baseline, 35% of the subjects were offloaded with the non-removable devices, while 50% were offloaded using the removable devices. Only 25% of patients received IV BPs, and around 20% received oral BPs. Follow-up data regarding resolution was available in 219 patients. The resolution was defined as a time-point when the patient starts walking in either normal or orthotic footwear. For those who received BPs, median resolution time was significantly longer than patients not receiving it (12 mo *vs* 10 mo, $P = 0.005$). Resolution time was significantly more in both groups as compared to other studies. One of the reasons for this can be the definition of resolution used, which required the patient to be ambulant. Regarding prolongation of resolution time with BPs, the authors have speculated the following possible explanations: first, BPs may have been used only in patients who had more severe CF or in non-responders to offloading alone. Second, BPs must have been used only if the non-removable device was unavailable. It is very tough to infer much from the results provided by this study. No data were provided regarding skin temperature or any BTMs or the type of BP used.

In 2013, Bharath *et al*[68] were the first to compare the effects of two BPs in a prospective randomized comparative study of 45 type 2 diabetic patients with acute CF. Patients were randomized into two groups, Z and A receiving a single IV infusion of 5 mg zoledronate (diluted in 100 mL normal saline over 30 min) and oral alendronate 70 mg once weekly, respectively, until resolution of the disease. The complete clinical resolution of the disease process was defined as attainment of a temperature difference of $< 1^{\circ}$ F between two feet on two different occasions. Patients with serum creatinine $\geqslant$ 3 mg/dL or with a history of BP exposure were excluded. For patients in group Z, if serum creatinine was $\geqslant$ 2 mg/dL, the dose of zoledronate was reduced to 2.5 mg. Patients in both the groups were offloaded using TCC. Forty patients completed the study

Table 1 Table of randomized controlled trials on bisphosphonates in acute Charcot foot

Ref.	BP	Duration (mo)	Jadad score	Subjects	Outcomes			
					Skin temp	Symptom score	BTM	Others
Jude *et al*[69]	Pamidronate	12	5	T: n = 21; 90 mg single IV infusion P: n =18; single IV infusion of NS	Significant reduction in both groups; more in T group at 4 wk (P < 0.01)	Significant improvement in T group from 3 – 12 mo (P < 0.01)	BSALP: Significantly greater reduction in T group till 12 wk (P < 0.03) uDPD crosslinks: Significant reduction in T group at 4 wk (P < 0.01)	
Pitocco *et al*[70]	Alendronate	6	1	T: n = 11; 70 mg once a week orally C: n = 9; no pharmacological treatment	Significant reduction in both groups	Signification reduction in T group at 6 mo (P < 0.05)	1CTP and uHP: Significant reduction in T Group (P < 0.05) BSALP: Greater reduction in T group (P = 0.06)	
Pakarinen *et al*[71]	Zoledronate	12	4	T: n = 20; 3 IV infusion of 4 mg at one monthly interval P: n = 19; placebo				Median immobilization time: Significantly greater in T group (P = 0.02)

BP: Bisphosphonate; T: Treatment group; P: Placebo group; C: Control group; IV: Intravenous; BTM: Bone turnover marker; BSALP: Bone specific alkaline phosphatase; 1CTP: Carboxyterminal telopeptide of type 1 collagen; uDPD: Urinary dehydroxypyridinoline; uHP: Urinary hydroxypronline.

(five patients withdrew), and 30 achieved complete resolution (16 patients in group Z and fourteen in group A). Five patients in each group achieved partial clinical resolution. The mean number of days required for complete healing process was around 122 d in both the groups.

Randomized controlled trials

All randomized controlled trials (RCTs) related to the use of BPs in acute CF are described in Table 1. In 2001, Jude *et al*[69] reported a 12 mo double-blind randomized placebo-controlled trial including 39 diabetic patients with acute CF. At baseline, 21 patients received single 90 mg infusion of pamidronate over 4 h, whereas 18 patients received normal saline (placebo). All patients received standard care of foot immobilization. For the first 3 mo, all patients were followed up at 2-weekly intervals and thereafter at 6, 9, and 12 mo. At each visit, patients were assessed for clinical symptoms, and skin temperature was measured with an infrared thermometer. BTMs like bone-specific ALP and urinary dehydroxypyridinoline were measured at each visit. Skin temperature reduced significantly in both the groups with pamidronate group showing a greater reduction at 4 wk. It dipped further during the study period with no intergroup difference on subsequent visits. Both groups demonstrated symptom score improvement at 3 mo. Following this, the score remained unchanged in the control group over the next 12 mo, whereas the pamidronate group registered further improvement (P < 0.01). Bone-specific ALP showed a significant reduction in the pamidronate group when compared to placebo (P < 0.03) at 4 wk, and this was maintained for at least 12 wk. A similar trend was observed in urinary dehydroxypyridinoline at 4 wk (P < 0.01). Both the BTMs gradually increased towards baseline at 12 mo.

In an observer blinded RCT, Pitocco *et al*[70] studied the efficacy of alendronate in patients with acute CF. Eleven patients included in study group received alendronate 70 mg orally once a week while nine patients in control group received no pharmacological treatment. All patients were followed up for 6 mo and were offloaded using a TCC boot for the first 2 mo, which was followed by a pneumatic walker in the subsequent 4 mo. BTMs like serum 1CTP, serum bone ALP, and urinary hydroxyproline were measured at baseline and at 6 mo of follow up. All these markers showed a significant reduction in the alendronate-treated group when compared to control group (P < 0.05), except for bone ALP (P = 0.06). Dual-energy x-ray absorptiometry done at baseline and at 6 mo showed statistically significant improvement in bone mineral density of total foot (P < 0.05) and distal phalanxes (P < 0.01) in the alendronate group. Visual analogue scale score for pain improved significantly in the treatment group, with no improvement in the control group (P < 0.05). Skin temperature reduced significantly in both groups at 6 mo.

In 2011, Pakarinen *et al*[71] first studied the effect of zoledronate in 39 diabetic subjects with acute CF in a double-blind randomized placebo-controlled trial. Patients were randomly assigned into two groups receiving three IV infusions of either 4 mg zoledronate or a placebo at 1-mo intervals. Patients with previous BP exposure or severe renal insufficiency were excluded. All patients were initially treated with a non-weight bearing cast and were allowed partial weight bearing when the clinical signs of active CF process subsided. Complete weight bearing was allowed only when the temperature difference between the two feet was less than 1℃ for at least last 30 d with no evidence of edema or erythema. All patients were evaluated at baseline, at 2 to 4 wk intervals for the first 3 mo and then at 6, 9, and 12 mo. Finally, 35 patients who completed 12 mo follow-up were analyzed. The final endpoint of this study was median immobilization time, which was significantly longer in the zoledronate treated group as compared to the placebo group (27 wk *vs* 20 wk, $P = 0.02$). No information was given regarding BTMs or radiological findings at any point of time. During 12 mo follow-up, one patient relapsed in each group.

DISCUSSION

The main aims of treatment in acute CF are to relieve the patient of symptoms and to avoid complications, such as deformity and ulceration, thus preventing the progression to chronic CF. Immobilization and off-loading are the most important components of this treatment. Avoidance of repetitive microtrauma leads to the resolution of edema and swelling. Casting should be continued until the skin temperature difference between the two limbs becomes less than 2℃[16]. However, the basic pathogenesis in CF revolves around osteolysis, which leads to subsequent bone destruction, and immobilization does not address this directly. This creates space for the adjuvant therapy that can inhibit osteolysis and hence bone resorption. BPs, calcitonin, and denosumab are the anti-resorptive agents used to date in these patients. Among BPs, maximum evidence in the literature is available for pamidronate[72]. In a majority of case reports and series, pamidronate was shown to reduce the markers of activity of CF, like skin temperature, pain, edema, and BTMs. In the first RCT assessing the response of BP in acute CF, Jude *et al*[69] confirmed the beneficial effects of pamidronate in patients with acute CF. This RCT was of high methodological quality, as it was a double-blind, placebo controlled, multi-center study with proper mention about randomization process and statistical analysis. In a retrospective case-control study, Anderson *et al*[66] reported significant reduction in skin temperature and serum ALP in the pamidronate treated group. However, in a case series by Pakarinen *et al*[63], no difference was found for casting times when pamidronate was used along with conventional measures. Among other BPs, alendronate in a RCT was shown to reduce pain and BTMs significantly in acute Charcot neuroarthropathy[70]. Additionally, zoledronate, the most potent third generation BP, was surprisingly shown to prolong immobilization times of patients with acute CF[71]. The limitations of this particular RCT were its underpowered nature (due its small sample size) and the discrepancy in the immobilization times. The latest randomized comparative study evaluating the effects of zoledronate and alendronate concluded that both medications had the same response in terms of clinical resolution time and scintigraphic changes. When cost was taken into account, however, alendronate was much less expensive than zoledronate[68].

None of these studies have ventured into the effect of BP on long-term outcome measures like avoidance of ulcerations, deformities, and amputation. The evidence from the available studies is limited because of the non-uniformity in the agent used and heterogeneity in outcome measures. Most studies, except one of Jude *et al*[69], have methodological flaws like open randomization, lack of blinding, and statistically small sample size. In fact, only Jude *et al*[69] reported the power analysis.

CLINICAL IMPLICATION

Oral alendronate and IV pamidronate have been efficacious in relieving symptoms and controlling disease activity in patients with acute CF. Oral BPs need to be taken on an empty stomach and with a full glass of water (at least 240 mL) to avoid getting it stuck in the esophagus. The patient should remain in erect posture for at least 30-60 min. Oral BPs have very poor bioavailability, with < 1% of the drug being absorbed from gastrointestinal tract[73]. BP should be taken in the fasting state with avoidance of any food for 30-60 min after taking to prevent its absorption from decreasing further. Retained gastric contents in patients with gastroparesis may also hamper absorption. Contraindications to oral BPs include an inability to follow this strict protocol, any active esophageal pathology like achalasia, varices, or stricture, or any malabsorption disorder like celiac disease, Crohn's disease, or post gastric bypass surgery[74]. For patients who cannot tolerate oral BPs, IV BPs can be an alternate option. IV BPs are known to cause acute phase reactions leading to flu-like illness in around 10%-30% of patients receiving their first infusion[74]. This can be taken care of by oral acetaminophen.

Vitamin D deficiency, which is common in the diabetic population, should be treated before giving BPs. In patients with renal insufficiency, caution should be exercised while using BPs, especially if glomerular filtration rate < 30-35 mL/min[74]. This is particularly true when given by rapid IV infusion, as it can aggravate or lead to renal dysfunction. Intranasal calcitonin can be an attractive option to treat acute CF in this group of patients. Moreover, BPs like zoledronate[75] and al-

endronate[76] have been linked with the occurrence of atrial fibrillation. HORIZON Pivotal Fracture Trial has shown a statistically significant increase in the incidence of serious atrial fibrillation in patients treated with zoledronate[75]. However, a large population-based study has refuted these findings[77].

As CN usually develops in diabetic patients with disease duration of more than 10 years, they are also expected to have gastroparesis, nephropathy, coronary artery disease, and various other complications. The above side-effects and contra-indications should be kept in mind while treating such patients with BPs.

CONCLUSION

The meteoric rise in the prevalence of DM has made it the most common cause of CN affecting foot and ankle. In a majority of the studies related to use of BPs in acute CF, pamidronate has been shown to reduce the markers of Charcot activity like skin temperature, pain, edema, and BTMs, but the quality of evidence is weak. Therefore, BPs can be considered as an adjuvant treatment option for acute CF.

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MINIREVIEWS

Reversibility of diabetes mellitus: Narrative review of the evidence

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Abstract

The global disease burden of diabetes mellitus is high. It is well-established that prediabetes is reversible but it is unclear whether diabetes is reversible once it has been diagnosed. The objective of this narrative review is to review the evidence of reversibility of diabetes mellitus and stimulate interest in prolonged remission as a treatment target. The current evidence for bariatric surgery is stronger than intensive medical management and the evidence is stronger for type 2 diabetes patients compared with type 1 diabetes patients. It is also unclear whether non obese diabetes patients would benefit from such interventions and the duration of diabetes before diabetes become irreversible. Further research is needed in this area especially with regards to the subgroup of diabetes patient who will benefit from these interventions and the long term safety and efficacy remains unknown especially with intensive medical management.

Key words: Diabetes; Reversibility; Remission; Bariatric surgery; Obesity

Core tip: Diabetes mellitus is potentially reversible especially with bariatric surgery. Intensive medical management is promising but the evidence is weaker. The subgroup that is likely to go into prolonged remission is those with insulin resistance, short duration of diabetes and obesity. Further research is needed to identify those that can go into remission and how to use intensive medical management to achieve this.

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INTRODUCTION

The number of adults with diabetes in the world has increased from 108 million in 1980 to 422 million in 2014 due to rise in prevalence, population growth and

ageing[1]. Diabetes Mellitus has been projected to become the 7th leading causes of death in 2030[2]. It has been estimated that the direct medical costs of diabetes to the world is more than United States $827 million[2]. It is well-established that prediabetes is reversible[3-7], but it is unclear whether diabetes is reversible once it has been diagnosed.

Type 2 diabetes mellitus is potentially reversible[8]. A better term to use would be remission which is defined to be achieving glucose level below the diabetic range in the absence of active pharmacologic or surgical therapy[9]. It can further be divided into partial or complete and if complete remission lasts for more than 5 years, it would be considered as prolonged remission[9]. In community settings, in the absence of bariatric surgery, the 7-year cumulative incidence of partial, complete or prolonged remission was found to be 1.47% (1.40%-1.54%), 0.14% (0.12%-0.16%) and 0.007% (0.003%-0.020%)[10] which is very low.

In this narrative review, the evidence of reversibility of diabetes mellitus will be reviewed in light of new studies recently published. This can help stimulate interest in prolonged remission as a treatment target for patients with established diabetes.

BARIATRIC SURGERY

There are several systematic reviews on the impact of bariatric surgery on diabetes mellitus[11-13] and some have distinguished between type 1 diabetes[14-16] and type 2 diabetes[17-20] (Table 1). The percentage of diabetes remission after bariatric surgery is estimated to be 76.8%[12] to 92%[11]. However, the exact physiological and molecular mechanisms behind diabetes remission after bariatric surgery remains incompletely understood[21,22]. (Figure 1) There are several reviews that looked at the role of bariatric surgery in managing diabetes mellitus[23-26] and the mechanism behind reversibility of type 2 diabetes mellitus[27,28] All agree that diabetes remission can be an important outcome to look at after bariatric surgery and there are many risk prediction models which can predict diabetes remission[29].

Besides diabetes remission, bariatric surgery may also reduce inflammation[30,31], improve renal function[31], reduce cardiovascular risk[32] and reduce microvascular and macrovascular complications[33]. The impact of bariatric surgery on all these remains incompletely understood.

Even if the evidence is strong for remission of diabetes after bariatric surgery, it is unlikely to be advocated at the population level due to the high cost and lack of surgeons well trained to perform bariatric surgeries. Furthermore, the indication for bariatric surgery is currently for patients with a body mass index above 35 kg/m^2 or between 30 and 35 kg/m^2 with inadequate glycemic control despite optimal medical treatment[34]. This would not benefit diabetes patients who are non-obese (body mass index < 30 kg/m^2) and the remission rate has been shown to be much lower in non-obese diabetes patients[18].

PANCREAS TRANSPLANTATION AND ISLET CELL TRANSPLANTATION

A recent review found that there is a need for multicenter randomized trials in pancreas transplantation to define clearly the efficacy, risks, and long term benefits due to lack of high quality evidence[35]. The indications for pancreas transplantation alone are in patients with severe metabolic complications, incapacitating problems with exogenous insulin therapy and failure of insulin based management to prevent acute complication[35]. It would not be to induce diabetes into remission. A systematic review on islet cell transplantation for type 1 diabetes mellitus has also concluded that there is low to very low quality evidence for all outcomes of interest such as remission of diabetes[36].

The next question to ask is whether pancreas transplantation is able to reverse complications of diabetes such as diabetic nephropathy. A recent study has demonstrated that diabetic nephropathy may be reversible after pancreas transplantation[37] that is contrary to current thinking. Further research is needed to look at whether it is possible to reverse diabetes and/or its complications after pancreas or islet cell transplantation.

INTENSIVE MEDICAL MANAGEMENT

There are relatively fewer studies on non-surgical remission of diabetes mellitus. A randomized controlled trial found that 40.7% of patients with type 2 diabetes for less than 3 years had complete or partial remission at 12 mo[38]. A cluster-randomized trial found that primary care-led weight management achieved a remission rate of 46% at 12 mo in patients with type 2 diabetes for less than 6 years[39]. A retrospective observational study of obese patients with type 2 diabetes found that 4.6 % achieved partial or complete diabetes remission after a 12-wk intensive program for diabetes weight management[40]. These studies did not look at the long term effectiveness of such intervention of the remission of type 2 diabetes mellitus and whether the same effect could be seen in patients with type 1 diabetes, non-obese diabetes patients or those with longer duration of type 2 diabetes.

Further research is needed to evaluate the long term effectiveness and safety of intensive medical management before recommending this but the results seem promising.

CLUSTERS OF DIABETES MELLITUS

A recent study has identified 5 replicable clusters of adult-onset diabetes with different disease progression and risk of diabetes complications[41]. The 5 clusters are

Table 1 Bariatric surgery systematic reviews

Ref.	Type of diabetes	No. of studies included	Remission percentage (95%CI)
Chang *et al*[11], 2014	Not specified	164 (37 randomized clinical trials and 127 observational studies)	Randomized clinical trials: 92% (85%-97%) Observational studies: 86% (79%-92%)
Buchwald *et al*[12], 2004	Not specified	136	76.8% (70.7%-82.9%)
Gloy *et al*[13], 2013	Not specified	11	59.90%
Ashrafian *et al*[14], 2015	Type 1 diabetes mellitus	27	Weighted mean decrease in insulin requirement: 44.5 units 78.1% (73.8%-82.3%)
Chow *et al*[15], 2016	Type 1 diabetes mellitus	13	Weighted mean total daily insulin requirement decreased from 98 +/- 26 IU/d to 42 +/- 11 IU/d
Mahawar *et al*[16], 2016	Type 1 diabetes mellitus	15	Not reported
Buchwald *et al*[17], 2009	Type 2 diabetes mellitus	621	76.2% insulin free
Baskota *et al*[18], 2015	Type 2 diabetes mellitus	10	61.8% medication free
Goh *et al*[19], 2017	Type 2 diabetes mellitus	24	Remission rate Duodenal-jejunal bypass: 20%-40% Duodenal-jejunal bypass with sleeve gastrectomy: 79%-93% Duodenal-jejunal bypass sleeve: 62.5%-100% Ileal interposition with sleeve gastrectomy: 47%-95.7%
Yan *et al*[20]	Type 2 diabetes mellitus	6	Type 2 diabetes mellitus remission rate for roux-en-y gastric bypass *vs* medical treatment: OR: 76.4 (95%CI: 20.7-281.7)

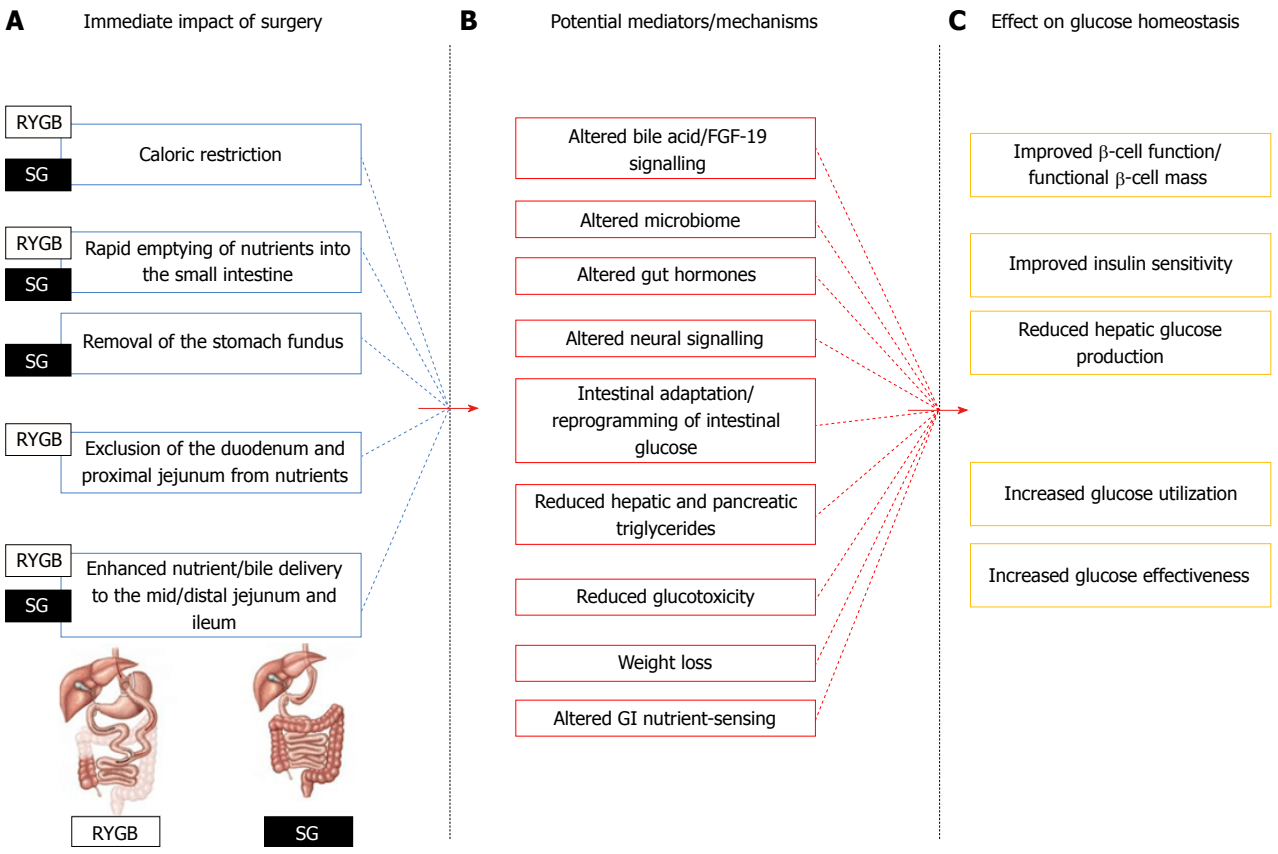

Figure 1 Potential mechanisms contributing to improved glycaemia after roux-en-Y gastric bypass and vertical sleeve gastrectomy. A: Immediate effects of improved glycaemia after roux-en-Y gastric bypass and vertical sleeve gastrectomy due to anatomical changes; B: Potential mediators/mechanisms involved. Cross talk occurs among these factors; C: Effects on glucose homeostasis[21]; RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy.

severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD)[41](Table 2).

Of the 5, it would be interesting to see which are more likely to go into prolonged remission with either bariatric surgery or intensive medical intervention so that clinicians can better define their treatment end-goals and treat accordingly. Based on insulin resistance, it would likely be SIRD, MOD and MARD that could go

Table 2 Subgroups of adult-onset diabetes

Subgroups	Body-mass index	Metabolic control	Insulin deficiency/resistance
Severe autoimmune diabetes	Relatively low	Poor	Insulin deficiency
Severe insulin-deficient diabetes	Relatively low	Poor	Insulin deficiency
Severe insulin-resistant diabetes	High	Fair	Insulin resistance
Mild obesity-related diabetes	High	Fair	Insulin resistance
Mild age-related diabetes	Relatively low	Fair	Insulin resistance

into prolonged remission.

Researchers may want to collect baseline data on glutamate decarboxylase antibodies, age at diagnosis, body mass index, glycated haemoglobin, and homeostatic model assessment 2 estimates of β-cell function and insulin resistance in future studies.

CONCLUSION

Diabetes Mellitus especially type 2 diabetes can go into prolonged remission *via* bariatric surgery or intensive medical therapy. The current evidence for bariatric surgery is stronger than intensive medical management but intensive medical management is likely to have a greater impact in type 2 diabetes management. More research is needed to understand the mechanism behind prolonged remission and to identify the group of diabetes patients that will benefit the most from such interventions.

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ORIGINAL ARTICLE

Retrospective Study

New-onset diabetes after kidney transplantation: Incidence and associated factors

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Abstract

AIM

To determine the incidence and associated factors of new-onset diabetes after transplantation (NODAT) in a Portuguese central hospital.

METHODS

This single-center retrospective study involved consecutive adult nondiabetic transplant recipients, who had undergone kidney transplantation between January 2012 and March 2016. NODAT was diagnosed according to the criteria of the American Diabetes Association. Data were collected from an institutional database of the Nephrology and Kidney Transplantation Department (Santa Maria Hospital, Lisbon, Portugal) and augmented with data of laboratorial parameters collected from the corresponding patient electronic medical records. Exclusion criteria were preexisting diabetes mellitus, missing information and follow-up period of less than 12 mo. Data on demographic and clinical characteristics as well as anthropometric and laboratorial parameters were also collected. Patients were divided into two groups: With and without NODAT - for statistical comparison.

RESULTS

A total of 156 patients received kidney transplant

during the study period, 125 of who were included in our analysis. NODAT was identified in 27.2% of the patients (*n* = 34; 53% female; mean age: 49.5 ± 10.8 years; median follow-up: 36.4 ± 2.5 mo). The incidence in the first year was 24.8%. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation, and 76.5% of the patients developed NODAT in the first 3 mo. In the group that did not develop NODAT (*n* = 91), 47% were female, with mean age of 46.4 ± 13.5 years and median follow-up of 35.5 ± 1.6 mo. In the NODAT group, the pretransplant fasting plasma glucose (FPG) levels were significantly higher [101 (96.1-105.7) mg/dL *vs* 92 (91.4-95.8) mg/dL, *P* = 0.007] and pretransplant impaired fasting glucose (IFG) was significantly more frequent (51.5% *vs* 27.7%, *P* = 0.01). Higher pretransplant FPG levels and pretransplant IFG were found to be predictive risk factors for NODAT development [odds ratio (OR): 1.059, *P* = 0.003; OR: 2.772, *P* = 0.017, respectively].

CONCLUSION

NODAT incidence was high in our renal transplant recipients, particularly in the first 3 mo posttransplant, and higher pretransplant FPG level and IFG were risk factors.

Key words: New-onset diabetes after transplant; Incidence; Kidney transplantation; Impaired fasting glucose; Immunosuppression

Core tip: New-onset diabetes mellitus after transplantation (NODAT) is a major complication of kidney transplant. The aim of this study was to evaluate the incidence and associated factors of NODAT among kidney transplant recipients in a single center. A total of 125 patients transplanted at Santa Maria Hospital (Lisbon, Portugal) were assessed, and NODAT was identified in 27.2%. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation and most patients (76.5%) developed NODAT in the first 3 mo posttransplant. Higher pretransplant fasting plasma glucose level and pretransplant impaired fasting glucose were predictive risk factors for NODAT development.

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INTRODUCTION

New-onset diabetes after transplantation (NODAT) is a frequent metabolic complication of kidney transplantation, and associated with increased morbidity and mortality[1,2]. However, due to the absence of a standard definition of NODAT, it has been difficult to determine a reliable incidence rate. The first International Consensus Guidelines published in 2003 for the diagnosis and management of NODAT were updated in 2014 and advocate the World Health Organization (WHO) and American Diabetes Association (ADA) criteria for the diagnosis of diabetes mellitus (DM) and impaired glucose tolerance (IGT)[3,4]. Recent studies using these criteria found incidences of NODAT to be 7%-30% in the first year after transplant[5-8].

Increased insulin resistance and impaired insulin production are likely to contribute to the development of NODAT[2]. Both traditional type 2 DM and transplant-related risk factors affect this condition[9]. The NODAT risk factors can be categorized into three groups: Non-modifiable, modifiable and potentially modifiable[10]. The non-modifiable factors include age, race/ethnicity, family history of DM, male recipient sex, the presence of certain human leukocyte antigens (HLAs; such as HLA A30, B27 and B42), increased HLA mismatches, donor-recipient mismatch, deceased donor kidney, male donor sex and history of acute rejection[10]. Polycystic kidney disease may confer an increased risk of NODAT, although results of the related studies remain conflicting[11]. On the other hand, the modifiable risk factors comprise obesity and type of immunosuppressive agents used to prevent or treat rejection. Finally, the potentially modifiable risk factors include pretransplant impaired fasting glucose (IFG) or IGT, and infection with hepatitis C or cytomegalovirus (CMV)[10].

The aim of this study was to evaluate the incidence of NODAT and its associated factors among kidney transplant recipients who were treated in a transplant center of a central Portuguese hospital.

MATERIALS AND METHODS

This is a single-center retrospective study of consecutive adult nondiabetic patients, who underwent kidney transplant between January 2012 and March 2016 at Santa Maria Hospital, Lisbon, Portugal. Data were collected retrospectively from an institutional database created by the Nephrology and Kidney Transplantation Department and completed with data for laboratorial parameters collected from the respective patients' electronic medical records, in agreement with our institutional ethical recommendations.

Inclusion and exclusion criteria

NODAT was diagnosed according to the ADA criteria (2017), which involves the following: Symptoms of diabetes (*i.e.,* polyuria, polydipsia or unexplained weight loss) plus random plasma glucose of ⩾ 200 mg/dL; fasting plasma glucose (FPG) of ⩾ 126 mg/dL, with fasting defined as no caloric intake for at least 8 h; and 2-h plasma glucose of ⩾ 200 mg/dL during an oral glucose tolerance test (OGTT). IFG was defined as FPG

between 100 mg/dL and 125 mg/dL[3].

In the first 3 mo after transplant, glycated hemoglobin was not used as diagnostic criteria, since its validity can be affected by the processes of new hemoglobin synthesis and glycation in the posttransplant setting[12]. The OGTT is considered the gold standard for diagnosing NODAT, enabling the identification of more patients than FPG measurement alone; likewise, it allows for diagnosis of IGT[4]. However, in our kidney transplantation center, the OGTT is not routinely performed in transplant recipients. The NODAT diagnosis was established when the immunosuppressive therapy and kidney allograft were stable and in the absence of acute infections or other stress factors, in order to exclude patients who developed transient hyperglycemia in the early posttransplant period[4].

Data on demographic/clinical characteristics, anthropometric and laboratorial parameters included age at transplant, sex, race, weight, height, calculated body mass index (BMI), etiology of primary renal disease, pretransplant FPG, history of hepatitis C or CMV infection, acute rejection episodes, type of transplant (deceased or living donor), type of immunosuppressive drugs for induction and maintenance therapy, follow-up time, graft loss and death. Exclusion criteria were preexisting DM, missing information (*i.e.,* pretransplant FPG) and follow-up period of less than 12 mo. A total of 156 patients were transplanted during the study period, and 125 of these were eligible for the study.

Immunosuppression regimen

All patients received induction therapy, consisting of either basiliximab (an interleukin-2 receptor monoclonal antibody; Protocol A) or rabbit antithymocyte globuline (ATG; Protocol B). Prior to the transplant, all patients received tacrolimus at 0.2 mg/kg. For Protocol A, the patient was administered 20 mg basiliximab pretransplantation and at 4 d posttransplantation; these patients also received tacrolimus at 0.075 mg/kg every 12 h and mycophenolate mofetil (1500 mg pretransplantation, followed by 1000 mg every 12 h for 1 wk posttransplantation and then 500 mg every 12 h). For Protocol B, the patient was administered 1.5-2 mg/kg ATG pretransplantation; methylprednisolone (500 mg) before ATG and tacrolimus at 0.05 mg/kg every 12 h.

All patients received 500 mg methylprednisolone intraoperatively, followed by 1 mg/kg per day for 3 d postoperatively, with progressive tapering until reaching 25 mg/d by the end of the first month after transplant. The maintenance therapy comprised corticosteroids (prednisolone), tacrolimus and mycophenolate mofetil.

Statistical analysis

Data were analyzed with SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, United States). A biomedical statistician (Nilza Gonçalves, Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Portugal) reviewed the study's statistics. For comparative analysis, the patients were divided into two groups: With and without NODAT. For continuous variables, differences were analyzed using the *Mann-Whitney* test (nonparametric data) and Student's *t*-test (parametric data). For categorical variables, differences were analyzed using the χ^2 test. Multivariate analysis was performed to identify potential risk factors for NODAT by using a logistic regression test. Data were expressed as mean ± SD or median (minimum and maximum) for continuous variables and as percentage for categorical variables. $P < 0.05$ was considered significant.

RESULTS

A total of 125 patients were enrolled for the analysis (mean age: 46.9 ± 12.9 years; 51.2% male). The majority of our patients were Caucasian, and the median follow-up was 35.7 ± 15.1 mo. NODAT was identified in 27.2% [*n* = 34; 95% confidence interval (CI): 20.17%-35.59%] of the patients; the NODAT cases were 53% female and had mean age of 49.6 ± 10.8 years. The incidence of NODAT in the first year was 24.8% (95%CI: 18.06%-33.05%).

The median time to diagnosis was 3.68 ± 5.7 mo after transplantation, with the majority of patients (76.5%) developing NODAT in the first 3 mo. NODAT diagnoses at the follow-up intervals of 3-6 mo, 6-12 mo and after 12 mo were 5.9%, 8.8% and 8.8%, respectively. The median follow-up for the NODAT group was 36.4 ± 2.5 mo. In the group that did not develop NODAT (*n* = 91), 47% were female and the mean age was 46.0 ± 13.6 years. The median follow-up was 35.5 ± 1.6 mo, which was not significantly different from that of the NODAT group ($P = 0.774$).

Table 1 compares the clinical and laboratory parameters of patients who developed NODAT with those who did not (NODAT *vs* non-NODAT). During the follow-up period, 1 patient in the NODAT group and 2 patients in the non-NODAT group died. There was no graft loss in the NODAT group, as opposed to the 5 cases recorded for the non-NODAT group.

In the NODAT group, the pretransplant FPG levels were significantly higher [101 (96.1-105.7) mg/dL *vs* 92 (91.4-95.8) mg/dL, $P = 0.007$] and the occurrence of pretransplant IFG was significantly more frequent (51.5% *vs* 27.7%, $P = 0.01$). Furthermore, higher pretransplant FPG levels and pretransplant IFG occurrence were identified as predictive risk factors for NODAT development [odds ratio (OR): 1.059, $P = 0.003$; OR: 2.772, $P = 0.017$, respectively).

Patients diagnosed with NODAT were more frequently of African origin (29.4% *vs* 22%), presented a trend for higher age (49.6 ± 10.8 years *vs* 46.0 ± 13.6 years) and BMI (25.2 ± 4.0 kg/m^2 *vs* 24.5 ± 4.4 kg/m^2), as well as a higher frequency of hepatitis C infection (2.9% *vs* 1.1%), CMV infection (97% *vs* 93%), acute rejection (14.7% *vs* 8.8%) and deceased donor (100% *vs* 91.2%), although none of these parameters

Table 1 Clinical and laboratory parameters

	NODAT group	Non-NODAT group	*P*
No. of patients	34 (27.2%)	91 (72.8%)	
Age at transplant (yr)	49.6 ± 10.8	46.0 ± 13.6	0.165
Female sex	53% (18/34)	47% (43/91)	0.571
Race			
Caucasian	70.6% (24/34)	78% (71/91)	0.387
African	29.4% (10/34)	22% (20/91)	
Body mass index (kg/m^2)	25.2 ± 4.0	24.5 ± 4.4	0.418
Pre-transplant FPG (mg/dL)	101 (96.1-105.7)	92 (91.4-95.8)	0.007
Pretransplant IFG	51.5% (17/33)	27.7% (23/83)	0.01
Hepatitis C infection	2.9% (1/34)	1.1% (1/91)	0.472
CMV infection	97% (33/34)	93% (82/88)	0.672
Acute rejection	14.7% (5/34)	8.8% (8/91)	0.338
Type of transplant			
Deceased donor	100% (34/34)	91.2% (83/91)	0.106
Living donor	0% (0/34)	8.8 % (8/91)	
Follow-up (mo)	36.4 ± 2.5	35.5 ± 1.6	0.774

CMV: Cytomegalovirus; FPG: Fasting plasma glucose; IFG: Impaired fasting glucose; NODAT: New-onset diabetes after transplantation.

reached statistical significance. The most frequent etiology of end-stage renal disease was hypertensive nephropathy (n = 7) in the NODAT group and polycystic kidney disease (n = 17) in the non-NODAT group.

In the NODAT group, induction therapy comprised ATG in 6 patients and basiliximab in 28; in the non-NODAT group, 24 patients received ATG and 67 received basiliximab. No statistically significant difference was found between the two groups for the induction therapies used (P = 0.309). In both groups, maintenance therapy consisted of immunosuppression with corticosteroids, tacrolimus and mycophenolate mofetil. Of the 34 patients diagnosed with NODAT, 44.1% (n = 15) needed oral hypoglycemic agents, 26.5% (n = 9) needed insulin and 5.9% (n = 2) were administered combined therapy (insulin and oral hypoglycemic agents). In the remaining 23.5% of the patients (n = 8), diabetes was controlled with diet and exercise alone.

DISCUSSION

Kidney transplant, besides being more cost-effective than dialysis, improves patient survival[13]. Nevertheless, NODAT is a frequent complication of kidney transplantation and is associated with poorer outcomes, increased risk of infectious and cardiovascular complications and reduced rates of patient and graft survival[5,14].

The reported incidence of NODAT has varied broadly between studies, probably due to the use of diverse diagnostic criteria, intensity of routine screening and follow-up length[15]. Furthermore, variability in the immunosuppressive protocols used in different transplant centers could influence the calculated incidence rates of NODAT. For instance, it is known that tacrolimus is more diabetogenic than cyclosporine[16]. Recent studies using the WHO/ADA criteria reported that 7%-30% of nondiabetic kidney transplant recipients develop NODAT in the first year after transplant[5-8]. In our study, NODAT was diagnosed in 34 patients (27.2%), with an incidence of 24.8% in the first year after transplant. Therefore, our findings are in agreement with previous studies. NODAT occurrence reportedly peaks in the first 3-6 mo posttransplant[17,18]. Studies have also shown that the incidence is higher when higher dosages of immunosuppressive medications are used[17]. After the 3-6 mo period, the annual incidence of diabetes is comparable to that observed in pretransplant patients[17,18]. In the present study, the median time to diagnosis was 3.68 ± 5.7 mo, with the majority of patients (76.5%) developing NODAT in the first 3 mo, which is also consistent with the literature.

Multiple risk factors have been identified. In our study, higher pretransplant FPG levels and occurrence of pretransplant IFG were predictive risk factors for NODAT development. Other researchers have reported abnormal glucose metabolism as a NODAT risk factor. For example, Cosio *et al*[19] reported that high pretransplant glucose levels represent a risk factor for NODAT at 1-year posttransplant. The risk was shown to increase as pretransplant FPG levels rose. Among patients with pretransplant IFG in that study, 70% had hyperglycemia at 1 year (IFG 43% and NODAT 27%). The strongest risk factor for NODAT seems to be age[20]. NODAT development is 2.2 times more likely to occur in patients with age above 45 years[21]. Another independent risk factor for NODAT is obesity or overweight status. Previous studies have reported a relative risk of 1.4 and 1.8 for patients with BMI between 25-30 kg/m^2 and > 30 kg/m^2, respectively[22]. We also found a trend for higher age and higher BMI in the NODAT group.

African-Americans have a 2-fold risk of developing NODAT compared to Caucasians. This finding can be, at least partly, related to immunosuppressive agents' pharmacokinetics variation[15]. Hepatitis C and CMV infection are also associated with NODAT. Hepatitis C virus causes insulin resistance in the context of liver dysfunction, abnormalities in glucose metabolism and

pancreatic β cell dysfunction[23]. Similarly, lower median insulin release has been reported for patients with CMV infection, suggesting impaired pancreatic β cell function as a possible pathogenic mechanism[24].

History of acute rejection episodes requiring elevated doses of glucocorticoids, as well as the type of transplant (deceased donor), have also been implicated in risk of NODAT[22]. We found higher frequencies of African-origin individuals, hepatitis C infection, CMV infection, acute rejection and deceased donors in our NODAT group, as suggested in the literature; however, the differences did not reach statistical significance. The majority of NODAT patients in our study required treatment for diabetes, with most responding to oral hypoglycemic agents, followed by insulin, and few requiring combined therapy. Nearly a quarter of the patients were able to achieve diabetes control without medication, based on lifestyle modifications.

Some limitations exist in our study design that may impact the interpretation and/or generalization of our findings. This was a retrospective study with a relatively small sample, only reflecting a single center experience. Moreover, OGTT is not currently used in our center as a NODAT screening test, which is likely to lead to underestimation of its incidence in this cohort.

The incidence of NODAT in renal transplant recipients is high, particularly in the first 3 mo. Recognition of the associated factors may help to prevent this condition. Higher pretransplant FPG levels and occurrence of pretransplant IFG were predictive risk factors for NODAT development, indicating a need for periodical blood glucose screening in patients waiting for a transplant in order to identify those at risk. Using the same rationale as for type 2 DM, early identification of impaired carbohydrate metabolism in the posttransplant setting will allow implementation of lifestyle modifications in order to minimize progression to NODAT and its potentially severe complications.

ARTICLE HIGHLIGHTS

Research background

New-onset diabetes after transplantation (NODAT) is a common complication of kidney transplantation, correlated with poorer outcomes. Its incidence varies greatly between studies, and multiple risk factors have been associated with its onset.

Research motivation

Albeit a frequent complication of kidney transplant, very few studies of NODAT in the Portuguese population have been published.

Research objectives

To evaluate the incidence and associated factors of NODAT among kidney transplant recipients in a Portuguese hospital.

Research methods

Retrospective study of consecutive adult nondiabetic patients, who underwent kidney transplant between January 2012 and March 2016 in a central Portuguese hospital.

Research results

NODAT was identified in 27.2% of the kidney transplant recipients. The median time to diagnosis was 3.68 ± 5.7 mo after transplantation. Higher pretransplant fasting plasma glucose levels and occurrence of pretransplant impaired fasting glucose (IFG) were predictive risk factors for NODAT development.

Research conclusions

Periodical blood glucose screening in patients waiting for a kidney transplant is important to identify those at risk for and to minimize progression to NODAT and its potentially severe complications.

Research perspectives

Clinicians should be aware of NODAT risk factors, namely pretransplant IFG, to perform a tighter surveillance of patients in these conditions. Multicentric studies are required to investigate other risk factors possibly implicated in NODAT development.

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