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BANTAO Journal

Official Publication of the BANTAO Association

Incorporating Proceedings of the BANTAO Association

Published by: Balkan Cities Association of Nephrology, Dialysis, Transplantation and Artificial Organs

Printing: BANTAO, 2016

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BANTAO Journal 2016; 14(2): 53-59

BJ

BANTAO Journal

Review article

Preeclampsia: from Pathophysiology to Treatment

Enton Kaculini[1], Alma Idrizi[2], Marsida Duli[1], Alketa Koroshi[2], Alma Shehu[3], Nereida Spahia[2] and Myftar Barbullushi[2]

[1]Faculty of Medical Sciences, Albanian University, [2]Department of Internal Medicine, Service of Nephrology, University Hospital Center "Mother Teresa", [3]Medical Representative, Novartis Pharma, Tirana, Albania

Abstract

Preeclampsia is a multisystem disorder unique to human pregnancy and is its most common glomerular complication. It occurs in 2% to 8% of pregnancies and is a major contributor to maternal mortality worldwide.
Although the pathophysiology of this syndrome is not fully understood, many pathogenetic mechanisms are involved in this disorder.
The role of the placenta is crucial in the development of this disorder. Some pathogenetic mechanisms involved in this disease comprise defective deep placentation, autoantibodies to type-1 angiotensin II receptor, endothelial dysfunction, oxidative stress, platelet and thrombin activation, intravascular inflammation, and the imbalance between angiogenic and antiangiogenic factors which is thought to be one of the most crucial mechanisms.
Further understanding of the full picture could enhance our current knowledge of the pathogenesis of preeclampsia and improve its treatment.
Thus, based on specific biomarkers the diagnosis and subclassification of preeclampsia might be more accurate in identifying patients at risk, monitoring disease progression and providing effective interventions.

Keywords: preeclampsia, angiogenesis, sFlt-1, spiral arteries, metformin

Introduction

Preeclampsia is a multisystem disease of the widespread vascular endothelial malfunction that is unique to human pregnancy, depicted by decreased GFR, proteinuria, and hypertension after 20 weeks of gestation, which can progress to include coagulopathies and affect liver function (HELLP syndrome) as well as cause seizures (eclampsia). It is the most common encountered glomerular complication in pregnancy [1,2].

Epidemiology and risk factors

Preeclampsia occurs in 2% to 8% of pregnancies, the risk is highest in those with a past history of preeclampsia, with rates ranging from 15% to 65% depending on the gestation at onset and the severity of preeclampsia [3].
Preeclampsia is more common in first pregnancies and lowers in subsequent pregnancies. The risk of preeclampsia returns to that of the first pregnancy in women who have a new partner for successive pregnancies, implying that prior exposure to paternal antigens could be protective [4]. However, this may be also explained by a longer interpregnancy interval rather than a change of partners, with the incidence increasing after about 7 years between pregnancies [5].
Smoking reduces the risk of preeclampsia by one third, increases the risk of preterm labor, intrauterine growth restriction (IUGR) and placental abruption [6].
Although in the majority of cases there is no family history, the presence of preeclampsia in the first degree relative increases the risk of severe preeclampsia two- to four-fold, suggesting genetic factors likely contribute to the pathogenesis of this condition. The risk is increased in subsequent pregnancies in women with preeclampsia in a previous pregnancy. Trisomy 13 in the fetus is associated with a high risk of preeclampsia in the mother.

Pathogenesis

The pathogenesis of preeclampsia is complex. The placenta likely causes preeclampsia, with other maternal organs (e.g., kidney) amplifying the disease process.
Observations have shown that gestational hypertensive disorders are more likely to develop in women who:

- are exposed to chorionic villi for the first time;
- are exposed to a great number of chorionic villi, as with multiple gestations or hydatidiform mole;
- have preexisting conditions of endothelial cell activation or inflammation such as diabetes or renal or cardiovascular disease;

Correspondence to: Enton Kaculini, Faculty of Medical Sciences, Albanian University, Tirana, Albania;
Phone: 0035 56 72 74 83 05; E-mail: enton_1@hotmail.com

- have a genetic predisposition to develop hypertension during pregnancy [8].

Regardless of etiology, preeclampsia is characterized by the following pathophysiologic triad:

- vasoconstriction
- platelet activation with intravascular coagulation (usually local but occasionally disseminated)
- maternal plasma volume contraction.

This triad leads to further impairment of blood flow through the placenta as well as through the maternal kidneys, liver, and brain. It is unknown why these organs are most often affected in preeclampsia or why other vascular beds (e.g., gut) are unaffected, even in severe cases [4].

An imposing number of mechanisms have been proposed to explain the cause of preeclampsia:

1. Superficial placentation with insufficient remodeling of spiral arteries and the impaired shear stress response

After the implantation of the blastocyst into the endometrium the trophoblasts continue to invade the uterine endometrium until they reach the spiral arteries, and during this period they also differentiate into an endothelial-like cell type.

Then the trophoblasts start spiral artery remodelation by replacing the smooth muscle and endothelial cells. The transformation of the thick muscular arteries into high-capacity vessels as a consequence of the increase in vessel diameter and the creation of a high blood flow, low resistance regions, permits more blood flow to the uteroplacental unit and also reduce the ability of these vessels for vasoconstriction [9]. During normal pregnancy trophoblast cells invade not only the deciduas, but also one-third of the myometrial thickness [10]. Thus researchers suggest that an abnormal trophoblast may result in shallow placentation and insufficcient transformation of the spiral arteries leading to preeclampsia [11]. The deeper myometrial arterioles keep their endothelial lining and musculature, and their mean external diameter is only half that of corresponding vessels in normal placentas [12] (Figure 1).

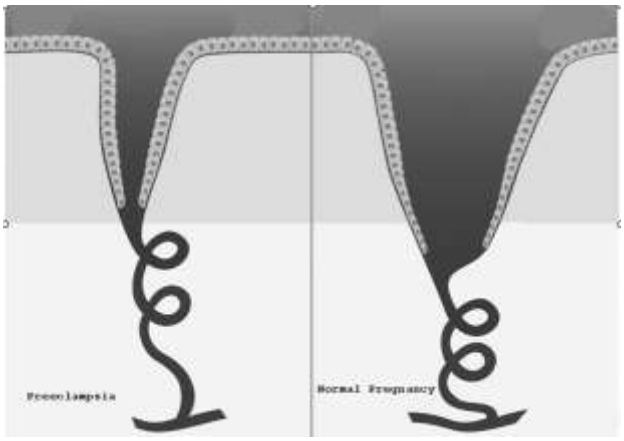

Fig. 1. The left side shows a preeclamptic situation in which there is insufficient extent and depth of remodeling compared to a normal pregnancy. The right side shows the endometrium in the second half of normal pregnancy with spiral arteries remodeled to a depth that penetrates the myometrium

Moreover, these nontransformed arteries are susceptible to atherosis, characterized by the presence of lipid-laden macrophages within the lumen, mononuclear perivascular infiltrate and fibrinoid necrosis [13,14].

The increased blood flow is essential for the developing embryo and the insufficient transformation of the spiral artery is linked with complications in pregnancy such as preeclampsia and intra-uterine growth retardation. In preeclampsia, this insufficient transformation of the spiral arteries into high capacity vessels is not complete and these arteries remain high resistance vessels which results in inadequate oxygen delivery, causing placental ischemia and infarction. The latter are responsible for the release of many factors that induce maternal vascular endothelial dysfunction. Probably, placental ischemia alone is not sufficient to cause preeclampsia because IUGR, also characterized by failure of physiological transformation of spiral arteries and placental insufficiency, does not often occur with preeclampsia [9,15].

Early preeclamptic changes include endothelial damage, accumulation of plasma components into vessel walls, proliferation of myointimal cells, and medial necrosis. Lipids are first accumulated in the myointimal cells and then inside the macrophages. These lipid-laden cell changes were referred to as atherosis by Hertig in 1945. In addition, it is thought that this acute atherosis identifies a group of women at increased risk of later atherosclerosis and cardiovascular disease [8,16].

Diminished perfusion and a hypoxic environment eventually lead to release of placental debris or microparticles that trigger a systemic inflammatory response [17, 18].

The effect of shear stress, besides trophoblast abnormalities, can also affect the spiral arteries' remodeling. The lumen of uterine arteries increases prior to completion of placentation.

There is also blood flow change in uterine arteries throughout the first few weeks of pregnancy, and after the remodeling of the spiral arteries into low-velocity and high flow comparments, the reduction of the downstream resistance (spiral arteries) increases blood flow velocity in afferent (radial and arcuate) arteries, causing high shear stress on the arterial wall [19]. This shear stress induced by the flow is a modulator of vascular tone in isolated arteries from normal pregnant women, mediated by nitric oxide (NO) production [20] resulting in vasodilatation, further lowering the uterine vascular resistance and normalizing the shear stress on the arterial wall [19]. In preeclampsia there is impaired shear stress-mediated NO release, which could play a role in vasoconstriction and increased vascular resistance, which

might also further impair the uteroplacental blood flow in this condition [21,22].

2. Hypoxia and trophoblast invasion: the role of HIF-1α and TGF-β3 in preeclampsia

During the early phases of implantation, the gestational sac is in a low oxygen tension medium, which favors trophoblast proliferation. Trophoblasts anchor the blastocyst to the endometrium, and also fill in the tips of the spiral arteries within the deciduas [23]. After the lacunae formation within the syncytiotrophoblast, these lacunae fuse with each other to create the intervillous space. The oxidative stress generated from the initial burst of blood inside this intervillous space due to high oxygen tension, promotes trophoblast differentiation from a proliferative to an invasive phenotype. These differentiated trophoblasts go deeper into the decidua, reaching into the superficial myometrium and making easier the physiological remodeling of spiral arteries. This means that the initial phase of placentation occurs under conditions of relative hypoxia [24].
Hypoxia-inducible factor-1 (HIF-1) is a key regulator of the cellular response to low oxygen tension and has an important role maintaining the oxygen homeostasis. It is a heterodimeric transcription factor made up by two subunits, α and β. While HIF-1β is always active, HIF-1α is oxygen-sensitive, which is quickly inactivated and degraded in normoxia [25].
In early gestation, there are high levels of HIF-1α in a low-oxygen placental milieu. These levels fall at around 9 weeks of gestation, when placental oxygen levels increase, suggesting an important role of HIF-1α in the placental development and function [26]. This early placental development is characterized by hypoxic environment and increased HIF-1α expression, leading to TGF-β3 upregulation and inhibition of trophoblast invasion. The increase in placental oxygen levels, around 10-12 weeks of gestation in a normal placenta, downregulates HIF-1α expresion and restores the trophoblast-invasive capabilities. In preeclampsia there is failure to downregulate HIF-1α and its persistent expression induce high placental TGF-β3 expression resulting in inhibition of placental explant trophoblast differentiation and invasion. These abnormalities are characteristic for this disease [27].
In addition, endoglin, the membrane-bound protein that gives rise to its proteolytic product sENG (a truncated form of a TGF-β receptor), is upregulated by HIF-1α [28] and sENG can be induced by hypoxia in trophoblasts [29].
Persistent placental hypoxia, present in the preeclamptic placenta, induces also the expression of angiotensin II type I receptor agonistic autoantibodies (AT1-AA), which stimulate the production of soluble fms-like tyrosine kinase-1 (sFLT-1), soluble endoglin (sENG), and endothelin-1. These factors lead to endothelial dysfunction and clinical manifestations of preeclampsia [30].

3. The role of renin-angiotensin-aldosterone system and angiotensin II type I receptor agonistic autoantibodies

For a long time, the renin-angiotensin-aldosterone (RAA) system has been suspected to play a role in the pathogenesis of preeclampsia. Normal pregnancy is characterized by reduced vascular responsiveness to angiotensin II. Conversely, in pregnant women with preeclampsia there is an increase in AT-II sensitivity [31]. Genetic predisposition, maladaptive immune responses and environmental stimuli are held responsible for the decrease or increase sensitivity to AT-II in normal and preeclamptic pregnancies [32].
Also, plasma renin activity (PRA) in patients with preeclampsia is lower compared to that in women with normal pregnancy [33]. Renin, the key enzyme that cleaves angiotensinogen precursor to angiotensin I is released in response to a decrease in blood pressure and/or perfusion to the kidney. Low PRA has been associated with increased circulatory volume [34] and it is thought that this is a compensatory response to hypertension in preeclampsia. There is also increased vascular responsiveness to AT-II in patients with preeclampsia [35].
In 1999, Wallucat *et al.* identified a subgroup of women with preeclampsia having [36] an agonistic autoimmune antibody to angiotensin II receptor type I (AT1-AA) in the circulation, but not present in healthy pregnant women. Since then, many studies have indicated that AT1-AA plays a role in the pathogenesis of preeclampsia. Stimulation of AT1 receptor by AT1-AA *in vitro* resulted in the inhibition of trophoblast invasiveness [37], a well-known characteristic of preeclampsia, and in the AT1 receptor activation in endothelial cells, vascular smooth muscle cells and mesangial cells [36]. Administration of these antibodies in pregnant rats leads to hypertension, proteinuria, glomerular capillary endotheliosis, increased production of sFLT-1 and sENG (leading to an anti-angiogenic state), placental abnormalities, and IUGR [38]. Data suggest that AT1 receptor activation by AT1-AA can induce calcium release in vascular smooth muscle cells and mediate the vascular changes in preeclampsia [39,40]. Some studies have shown that this autoantibody is present in the serum of more than 95% of women with preeclampsia and that its serum concentrations correlate with disease severity, which gives further support to the role of AT1-AA in the pathogenesis of preeclampsia [41].
Another interesting finding is that reduced uterine perfusion pressure in rats induces production of anti-AT1 autoantibodies, sFLT-1, TNF and endothelin-1, as well as hypertension and proteinuria. Also, the administration of TNF, IL-6 or IL-17 to pregnant rats is associated with hypertension, placental oxidative stress and increased AT1 activity, the effects of which can be counteracted by the administration of losartan (a selective angiotensin II type 1 receptor) or by B-cell depletion using rituxi-

mab [42]. Furthermore, anti-AT1 autoantibodies mediate hypertension during pregnancy through activation of complement C3 and production of antiangiogenic factors [43].
However, the underlying mechanism that leads to the production of AT1-AA in preeclampsia is still unknown and several controversies remain before one can accredit a pathogenetic role for AT1-AA in preeclampsia. First, the fact that the incidence of preeclampsia is greatest in the first and does not worsen in subsequent pregnancies is in contrast with an autoimmune basis for this disease. Second, AT1-AA is not specific to preeclampsia or to pregnancy; it also occurs in patients with graft rejection and accelerated hypertension [44]. And third, levels of aldosterone, which is downstream to angiotensin II signaling, are decreased in preeclampsia rather than being up-regulated [45].

4. Angiogenic imbalance in the pathophysiology of preeclampsia

Angiogenesis, the formation of new blood vessels from pre-existing ones, is essential for a successful pregnancy [46]. Defective angiogenesis in the pathogenesis of preeclampsia points toward an imbalance between proangiogenic factors, such as vascular endothelial growth factor (VEGF), placental growth factor (PLGF) and transforming growth factor-β (TGF-β), and antiangiogenic factors, such as soluble fms-like tyrosine kinase-1 (sFLT-1) and soluble endoglin (sENG) [4].
Experiments suggest that sFLT-1 and sEng neutralize their ligands acting like antiangiogenic agents lowering the serum concentration of VEGF, PLGF, and TGF-β, which shifts the angiogenic balance towards antiangiogenesis, and leads to endothelial damage triggering hypertension and proteinuria in up to 67% and 63% of patients, respectively [47,48].
The measured mRNA levels of the soluble VEGF receptor-1 (sFlt-1) are higher in the placentas of patients with preeclampsia than in those of healthy pregnant women. Many manifestations in preeclampsia have been reported to be induced by iatrogenic VEGF inhibition in humans supporting the role of sFlt-1 in the pathogenesis of preeclampsia [49-51]. Sera from women with preeclampsia showed antiangiogenic effects on endothelial tube formation assays (used as a test of angiogenesis), which could be reversed by addition of VEGF and PlGF. In pregnant animals high levels of sFlt-1 induced hypertension, proteinuria and glomerular capillary endotheliosis [49].
In preeclampsia, serum samples taken from the uterine vein show significantly higher levels of sFlt-1 than those taken from the antecubital vein, but not in normal pregnant women. Placenta is the main source of the elevated serum levels of sFlt-1 in preeclampsia [49]. Maternal plasma levels of sFlt-1 increase proportionally with the severity of the disease, and they are higher in early than in late preeclampsia [50]. Also, maternal plasma concentrations of sFlt-1 are increased before the clinical diagnosis of preeclampsia and decrease markedly after delivery [49].
A second antiangiogenic factor implicated in the pathogenesis of preeclampsia is soluble endoglin, a cell-surface co-receptor of TGF-β1 and TGF-β3 that induces migration and proliferation of endothelial cells. Suggestions of soluble endoglin involvement in the pathogenesis of preeclampsia include its higher maternal plasma concentrations in women with preeclampsia compared to healthy pregnant women, both before and at the time of clinical diagnosis; also these levels correlate with the severity of the disease [52,53].
Reduced uteroplacental blood flow [54,55], damage to the villous trees, syncytial shedding of antiangiogenic factors [56], oxidative stress [57], anti-AT1 autoantibodies [40], proinflammatory cytokines [38], excess thrombin [58] and hypoxia [59] have all been proposed to be responsible for the increased weight of the antiangiogenic state in preeclampsia (Figure 2).

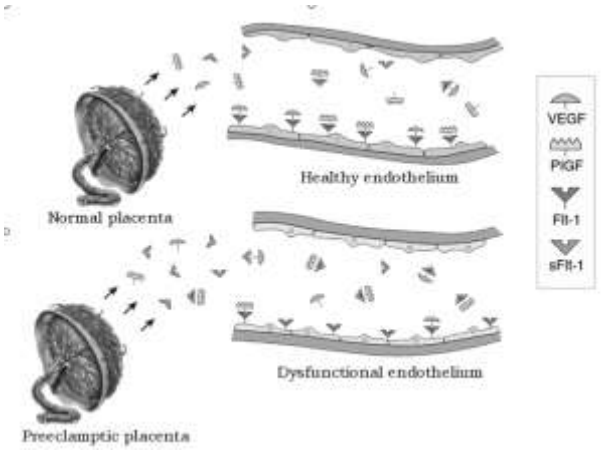

Fig. 2. sFlt-1 and sEng cause endothelial dysfunction by blocking VEGF and TGF-β1 signaling

5. The role of oxidative stress

Oxidative stress, the presence of reactive oxygen species in excess of antioxidant buffering capacity, is a prominent feature of preeclampsia. Oxidative stress is known to damage proteins, cell membranes, and DNA and is a potential mediator of endothelial dysfunction. Maternal blood enters into the intervillous space at higher pressure and faster rate because of the impaired arterial remodeling of the spiral arteries. This exposes the placental villi to fluctuating oxygen concentrations, leading to oxidative stress and activation of nuclear factor- κB, a transcription factor central to the inflammatory response [60].

Kidney manifestations of preeclampsia

The pathologic swelling of glomerular endothelial cells in preeclampsia is known by the term glomerular endotheliosis. The primary injury is specific to endothelial cells. The glomeruli are enlarged and swollen primari-

ly as a result of hypertrophy of the endothelial cells, which occlude the capillary lumina, giving the appearance of bloodless glomeruli [61]. Fibrinogen and fibrin deposits are found within and under the endothelial cells, and electron microscopy shows vacuolization and loss of glomerular endothelial fenestrae [62]. The podocyte foot processes are intact at early stages of disease, a finding not commonly seen in other nephrotic diseases. There are also changes described in the afferent arteriole, including atrophy of the macula densa and hyperplasia of the juxtaglomerular apparatus [63].

Until recently, glomerular endotheliosis was considered a pathognomonic finding in preeclampsia, but new studies have shown that mild glomerular endotheliosis also occurs in pregnancy without preeclampsia, particularly in gestational hypertension.

This suggests that the endothelial dysfunction of preeclampsia may in fact be an exaggeration of a process present in normal healthy pregnancies [64]. Both GFR and renal blood flow are low in preeclampsia as compared to normal pregnancy, the former more than the latter, leading to a 25% reduction in filtration fraction. Due to glomerular capillary endotheliosis there is GFR reduction as a result of both a decrease in renal blood flow and a decrease in the ultrafiltration coefficient (Kf). Renal blood flow decreases as a result of high renal vascular resistance, mainly because of increased afferent arteriolar resistance.

Even though acute renal failure can occur in preeclampsia, the only renal manifestations of disease are typically proteinuria (with bland urinary sediment) with renal sodium and water retention. Because GFR increases in pregnancy, serum creatinine levels in preeclampsia may still appear relatively normal. Proteinuria is generally nonselective and can appear late in pregnancy. In preeclampsia, the podocytes are normally intact, so the etiology of proteinuria is uncertain. There is impaired excretion of sodium and uric acid. The latter is an important marker of preeclampsia and is the cause of hyperuricemia. In contrast to normal pregnancy, preeclampsia is often associated with hypocalciuria [3, 9].

Treatment and Novel Therapies for Preeclampsia

Extracorporeal Removal of Soluble Fms-Like Tyrosine Kinase 1

Recent advances in understanding the pathophysiology of preeclampsia have shown new potential therapeutic targets. Interfering with the production or signaling of sFlt1 may ameliorate the endothelial dysfunction of preeclampsia, allowing to postpone more safely the delivery [7].

Currently there are no target therapies to prevent the clinical manifestations and prolong pregnancy in preeclampsia. New therapies aimed at circulating sFlt-1 can improve the signs and symptoms of preeclampsia and probably lengthen the time of pregnancy in women presenting with very preterm (gestational age <32 weeks) preeclampsia. The challenge for the researcher is to find effective therapies that would be safe for both mother and baby. Instead of administering an agent to the mother, a safe form of therapeutic apheresis would be the extracorporeal removal of sFlt-1 using a selective adsorption column, which would create a concentration gradient and augment its removal from maternal circulation. The depletion of circulating sFlt-1 was found in some trials to prolong pregnancy in women with very preterm preeclampsia. These therapies were well tolerated by both mother and baby, but further clinical studies may be required to determine if this intervention can safely prolong pregnancy in women with very preterm preeclampsia [65].

Metformin as a prevention and treatment of preeclampsia

Some researchers have found that a cheap drug already used to treat diabetes can block the release of toxins from the placenta when preeclampsia is present. This drug, called metformin, has the potential to prevent or treat preeclampsia, and is safe in pregnancy. Metformin is also reported to inhibit hypoxic inducible factor 1α by reducing mitochondrial electron transport chain activity which is upregulated in preterm preeclamptic placenta. Given the mitochondria appears to positively regulate sFlt-1 and sENG secretion, it is hypothesized that preeclamptic placentas might have increased mitochondrial electron transport chain activity. Metformin reduced fms-like tyrosine kinase 1 and soluble endoglin secretion from primary human tissues, possibly by inhibiting this mitochondrial electron transport chain [66].

Metformin reduces VCAM-1 expression on endothelial cells. Endothelial dysfunction is associated with increased VCAM-1 expression in the endothelium. VCAM-1 is an adhesion molecule that is expressed on the luminal surface of blood vessels and plays a major role in the recruitment of circulating blood cells enhancing the inflammation. Preeclampsia is also associated with increased circulating TNFα, which is a proinflammatory cytokine that upregulates VCAM-1 [67-69]. The administration of metformin significantly reduced TNFα-induced VCAM-1 expression, which suggests that it may have effects on decreasing endothelial dysfunction [66].

A trial involving 40 women showed that metformin at a dose of 1.7 g per day was associated with a significantly lower rate of preeclampsia than the rate among women who received placebo [70].

Conclusions

In conclusion, metformin reduces endothelial dysfunction, enhances vasodilation in omental arteries, induces angiogenesis and also seems to heal injured blood vessels. Thus, metformin may be a novel preventative or

therapeutical agent for preeclampsia and has the potential to reduce the disease, which affects many pregnant women around the world each year, causing many to deliver premature babies. However, further clinical studies are warranted.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 60-63

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Review article

Nephropathology: A Cornerstone for Understanding and Estimation of Recent Advances in Glomerular Diseases

Cristina Capusa[1,2], Ana-Maria Mehedinti[2], Sabine Leh[3] and Hans-Peter Marti[3]

[1]Nephrology Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, [2]"Dr Carol Davila" Teaching Hospital of Nephrology, Bucharest, Romania, [3]Department of Clinical Medicine, Renal Research Group, Haukeland University Hospital, University of Bergen, Bergen, Norway

Abstract

The developments in the field of kidney pathology are major objectives for nephrology worldwide, since the histopathologic diagnosis is a cornerstone for all glomerulopathies (either primary or secondary related to systemic diseases-for tubulointerstitial and vascular lesions as well as renal allograft nephropathy). Moreover, the correct interpretation of kidney tissue samples is a challenge for pathologists too. Consequently, a new subspecialty - nephropathology, was accepted by many medical schools in various universities, while dedicated scientific meetings, journals and websites were also created. In the following few pages, a short overview on the history, classic and novel meanings of the renal pathology for the understanding of glomerular pathophysiology will be discussed.

Keywords: chronic kidney disease, glomerulopathies, kidney histology, nephropathology, proliferative pattern

Introduction

Nephropathology (or renal pathology) represents a branch of pathology aimed to study and diagnose kidney diseases (both in native kidneys and renal allografts) by microscopic examination of biopsy-obtained renal tissue. It provides useful data for everyday clinical practice in nephrology, but it also contributes to the scientific research related to kidney diseases.

Primary and secondary glomerulopathies are prevalent causes of chronic kidney disease (CKD), which could affect around 10% of the general population and thus earning its place among chronic non-communicable diseases. Although not always recognized [1,2] CKD provides a significant burden on any health care system. Kidney biopsy certifies the diagnosis of glomerular diseases, thus allowing stratification of patients into specific disease sub-groups which is important for treatment purposes in order to possibly influence the outcome on an individual basis. In addition, it also provides tissue samples for transcriptomic, genomic and proteomic studies, finally meant to select novel biomarkers for the prevention, early detection and proposing new therapies for CKD.

Historical evolution of nephropathology

Even if the ancient roots of pathology could be tracked back to the Galen's period, the real dawn of this specialty can be traced in the fifteenth century, when the first case histories and autopsies were done [3]. However, only two centuries later, after the invention of the microscope in Holland, the seeds of renal pathology were seen (Figure 1). Marcello Malpighi was the first to study not only the gross anatomy, but also the microanatomy of the kidneys and discover a part of its functional unit (i.e. the nephron), which he called a "gland" attached to arteries [4]. Malpighi assumed that it is the place of urine formation through a process of filtration from blood, according to substances' size and shape [4,5]. Thus, it can be said that both renal pathology and renal physiology were born at that time. Despite this first step, the next three centuries brought rather meagre achievements in the field of kidney histopathology. For example, in the mid-nineteenth century, the proximal (William Bowman) and then the lower parts of nephron (Friedrich Henle) were described, new dyes became available to enrich the staining options, and the technique of paraffin embedding for tissues preservation was created (Edwin Klebs) [5]. Klebs was also the first who introduced the term of "glomerulonephritis".

During the same period, which can be designated as the pre-biopsy era (Figure 1), other important landmarks are the Richard Bright's basis of clinical nephrology (with his studies on kidney-related symptoms and the first attempt to classify kidney diseases), and the integration of clinical and pathologic findings which lead Sir Arthur Ellis to describe two types of nephritis (the first with glomerular hypercellularity and the second with glomerular sclerosis) [3,5]. Another forward move-

Correspondence to: Cristina Capusa, "Carol Davila" University of Medicine and Pharmacy - Nephrology Department at "Dr. Carol Davila" Teaching Hospital of Nephrology, 4 Calea Grivitei, sector 1, 010731, Bucharest, Romania; Phone: 0040-722-409832; Fax: 0040-21-3129226, E-mail: ccalexandr@yahoo.com

ment took place that time in the field of renal physiology by the work of Carl Ludwig who applied the laws of physics (hydrodynamics) to explain the glomerular filtration process of urine formation [4]. His results were confirmed and further developed by the micropuncture experiments of JT Wearn and AN Richards, which demonstrated a differential reabsorption of glomerular filtered solutes into the tubules, and hence put the basis of the modern renal physiology [4].

At the beginning of the 20th century, the first collaboration between a (renal) pathologist-Theodor Fahr and a clinician (treating internist)-Franz Volhard resulted in a new classification of kidney diseases driven by the combination of pathology findings and clinical signs of the

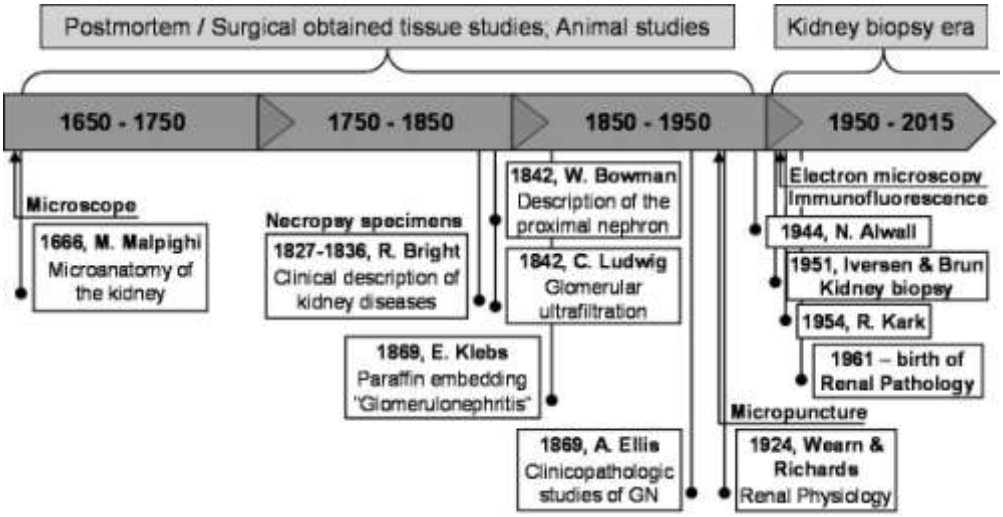

Fig. 1. The timeline of historical evolution of nephropathology as used in clinical routine

disease: nephrosis-i.e. the degenerative diseases, nephritides-i.e. inflammatory diseases, and chronic nephrosclerosis [5]. The successive progress regarding the techniques of tissues fixation, embedding, cutting, staining, microscopy, and image processing constantly yielded more accurate diagnostic tools [3], but the true turning point in the evolution of nephropathology is the introduction in practice of the percutaneous kidney biopsy around the mid-20th century (firstly used but not reported by Nils Alwall, then by Paul Iversen and Claus Brun, and finally refined by Robert Kark). Along with the almost simultaneous discover of immunofluorescence (Albert Coons and Melvin Kaplan, 1950) and the use of electron microscopy (firstly invented by Ernst Ruska and Max Knoll, 1931) in the medical field, this marked the entrance in the biopsy era [6]. One decade later, the nephrology was recognized as an independent specialty and the inaugural symposium on nephropathology was held, a fact that contributed to connect clinical nephrology and renal pathology as a synergistic entity [6]. From then onward, the rhythm of discoveries and new insights into the understanding of kidney disease pathogenesis (especially glomerular diseases) gathered speed in an unprecedented manner, leading to today's knowledge.

Nephropathology and established advances in glomerular pathology

Once introduced in the daily nephrological practice, kidney biopsy not only became the foundation of the great improvements in the diagnosis accuracy, but it also contributed to the deep understanding of disease mechanisms and provided important clues for the classification of kidney diseases, which opened the way to more efficient therapies. Light microscopy, immunofluorescence and electron microscopy are complementtary techniques and all concur to the precise description of the histopathologic changes in the kidney [7].

The understanding of glomerular pathology was the main benefit of the obtained knowledge from nephropathology. The diagnosis, pathogenetic insights, prediction of prognosis and therapeutic decisions of various nephropathies are stronger than in any other diseases relying on the microscopic assessment of the biopsy-extracted kidney tissue. Firstly, new histological patterns were observed and gradually characterized. Furthermore, during the late '60s and early '70s, the time-related classification of glomerulopathies (acute, subacute and chronic) was replaced by the morphologic classification, which was considered a great step forward because it assured the reproducibility of diagnosis and opened the pathway to a pathogenetic classification [8].

A good example is immunoglobulin A nephropathy (IgAN)-the most common primary glomerulonephritis worldwide, accounting for almost one third of cases [9]. It was initially described by Jean Berger and Nicole Hinglais in 1968 with the help of immunofluorescence which showed dominant mesangial deposits of IgA (with/without IgG) associated with moderate mesangial hypercellularity [10]. Since this first report, successive attempts of histological classifications and grading of

IgAN were made in order to obtain indices for predicting the outcome. Lee *et al*. (1982) and Haas *et al.* (1997) both proposed five-grade systems based on the spread of glomerular lesions (focal and segmental versus diffuse mesangial proliferation), the presence and spread of crescents, glomerular sclerosis and of the tubulointerstitial fibrosis [9]. Alamartine *et al*. (1990) developed a quantitative scoring system (the global optical score) ranging from 0 to 20, based on the sum of the glomerular, vascular, tubular and interstitial indices of injury [9]. However, none of these efforts were widely accepted, and the lack of consensus triggered the work of an international expert group to conceive a reliable and clinically significant, evidence-based new classification. The Oxford classification of IgAN is built on the MEST score [11]:

- mesangial hypercellularity-in ≤50% glomeruli=M0 and in >50%=M1;
- endocapillary proliferation-absent=E0 and present=E1;
- segmental glomerulosclerosis or adhesion-absent=S0 and present=S1;
- tubular atrophy and/or interstitial fibrosis-in <25% glomeruli=T0, in 26-50%=T1, and in >50%=T2.

The independent prognostic value of these four histological indices for IgAN patients was subsequently confirmed by numerous validation studies [9]. The only probable drawback of the Oxford classification is the lack of crescents from the scoring system (due to their low prevalence in the original investigated cohort [11]), since several studies found the extracapillary proliferation as an independent risk factor for the disease progression [12,13].

The contribution of electron microscopy should also be emphasized, as the histological hallmark of minimal change disease (one of the four major primary glomerulopathies which are clinically identified by the nephrotic syndrome), the diffuse foot process effacement, is visible only on the ultrastructural evaluation of kidney tissue. Moreover, this lesion was observed in other glomerular diseases associated with nephrotic syndrome too, thus leading to a higher awareness about the involvement of podocytes in glomerulopathies. The final result was the outlining of an array of glomerular diseases induced by altered podocyte functions, namely the podocytopathies: minimal-change disease nephropathy, focal and segmental glomerulosclerosis, diffuse mesangial sclerosis, and collapsing glomerulopathy [14,15].

Membranoproliferative glomerulonephritis (MPGN) is another example of glomerular disease, which understanding about classification and mechanisms closely followed the historical evolution of knowledge in the field of nephropathology. Firstly, it was identified as a descriptive pattern of glomerular injury observed on light microscopy and was included in the histopathologic classification of primary glomerulopathies published by Renee Habib in 1975 [16]. Further studies using electron microscopy were able to divide MPGN in three subtypes according to the location of electron-dense immune deposits inside the glomerular capillary wall [17]. More recently, the extensive evaluation by immunofluorescence found significant differences in the composition of immune deposits, strongly related to the etiopathogenesis of the disease, thus laying the foundation of the current classification into two major groups: immunoglobulin-mediated and complement-mediated MPGN [18]. This new proposed taxonomy has the advantages of being more pathophysiologically-oriented and providing important clues for the subsequent etiological diagnosis. Also, it allowed the surfacing of the C3 glomerulopathy (MPGN with deposition of C3 alone), a new disease entity due to inherited or acquired dysregulation of the alternative complement pathway, with potential implications for the therapeutic decisions [6,17].

However, since the glomeruli have limited possibilities of reaction to various injuries, the glomerular histopathologic changes usually overlap among different glomerulopathies irrespective of their etiology and even pathogenesis. Therefore, it is conceivable that a future step will be to incorporate serologic, genetic and molecular information to the clinical and histological findings, in a broad interdisciplinary effort to enhance the diagnosis and prognosis accuracy in the field of glomerulopathies [6].

Nephropathology and future advances in glomerular pathology

The most recent innovation in kidney biopsy assessment is the introduction of a computer-aided tool, by scanned slides and the setting up of a digital archive, which is easier to access and represents valuable stored information. The method seems also to enhance the performance of histological diagnosis, since in a recent study on whole slide images of 277 biopsies from the Nephrotic Syndrome Study Network (NEPTUNE) digital pathology repository, the digital technique was able to increase the accuracy of glomeruli counting, especially in cases with high number of glomeruli or high proportion of glomerulosclerosis (> 40%) [19].

Further ahead, the cooperation between classic nephropathology and the newest methodological advances in molecular biology (proteomics, metabolomics and transcriptomics) would be most probably the future in the field of glomerular pathology and kidney transplantation [6,20]. Laser capture microdissection and mass spectrometry-based proteomic analysis in biopsy specimens has already emerged as a valuable proteomic tool for the identification and subtyping of renal amyloidosis, with 100% reported specificity and sensitivity [21]. Also, the *in-situ* proteomics technology known as matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) applied in cases of minimal change disease and membranous nephropathy showed molecular changes expressed as different signals in control ver-

sus patients with the two glomerulopathies, suggesting the possibility to detect the proteomic "signature" of the disease [22]. MALDI-IMS, which can analyze either fresh frozen tissue or formalin-fixed paraffin-embedded samples, opens opportunities to discover new biomarkers with both diagnostic and prognostic value [23].

On the other hand, transcriptomics was used in conjunction with renal pathology as well. Thus, the quantitative genome-wide mRNA expression analysis from kidney biopsy specimens by microarrays technique (next generation sequencing) uncovered abnormalities in slit diaphragm and podocyte transcripts, which are different in primary focal and segmental glomerulosclerosis (FSGS) from minimal change disease [6]. The method also identified complex genetic mutations in the spectrum of FSGS and it can be expected to provide similar new insights for other hereditary glomerular diseases with various genes aberrations but common histological and clinical characteristics, like Alport syndrome [24]. The same is true for the kidney graft pathology, as a recent multicentre study reported a set of 13 genes which was an independent predictor of the risk to develop fibrosis at one year in kidney allograft recipients, with high predictive capacity (area under the curve 0.967), hence it was proposed to use this set of genes as an early predictor of the risk for progressive loss of graft function [20].

However, until now, the high costs of the novel methods of molecular biology and, more important, the high number and heterogeneity of proposed biomarkers hinder the routine clinical utility of proteomics, transcriptomics and genomics. Presently, the correlation of detailed clinical, laboratory, immunological and histopathological data remains the most reliable diagnostic tool, and the place of nephropathology in the management algorithm of patients with glomerulopathies is well established. For this reason, aiming to strengthen this subspecialty in our country, a series of courses in nephropathology for the trainees and young nephrologists have recently been initiated, the first of which just has ended few weeks ago and was highly appreciated by the attendees.

Acknowledgments: Funded through the European Economic Area Financial Mechanism 2009-2014, the Bilateral Relations Fund of the Programme RO14 "Research within Priority Sectors" - Contract No. 4BIL/19.09.2016.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 64-66

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Original article

Clinical Course of Children and Adolescents with Primary Vesicoureteral Reflux: A retrospective study of 958 patients

Abbas Madani[1], Yalda Ravanshad[2], Anoush Azarfar[3], Niloofar Hajizadeh[1], Nematollah Ataei[1], Zahra Adl[1], Zahra Pournasiri[4], Sepideh Bagheri[3], Sahar Ravanshad[5] and Elham Samazghandi[3]

[1]Department of Pediatric Nephrology, Children's Hospital Medical Center, Tehran University of Medical Sciences, [2]Clinical Research Development Center, Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [3]Department of Pediatrics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [4]Department of Pediatric Nephrology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [5]Department of Internal Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Introduction. Vesicoureteral reflux (VUR) is the most common pediatric urologic abnormality and since it can predispose to urinary tract infection and resultant kidney scar it is an important issue in pediatric nephrourology.
Methods. A retrospective chart review and follow-up of 958 patients with primary VUR was performed in the Children's Medical Center, Tehran, Iran.
Children with primary vesicoureteral reflux were included in the study and these parameters were studied: age, sex, clinical presentation, VUR grade, sonographic findings, DMSA changes, treatment modality (medical, surgical or endoscopic) and response to treatment, hypertension (presence/absence), urinary tract infection recurrence and development of new kidney scars in patients under medical treatment.
Results. VUR was more prevalent in girls. Sonography was unable to detect VUR in many cases. Presence of renal scars was strongly associated with degree of reflux. Medical management was effective in a substantial percentage of patients and they experienced full resolution of reflux. This was especially true for lower degrees of VUR. 17.6% of patients developed new kidney scars on follow-up which was associated with higher degrees of VUR. Hypertension and breakthrough urinary tract infection was an uncommon finding in our patients.
Conclusion. Medical management, which means using prophylactic antibiotics for prevention of urinary tract infection, is effective in many cases of VUR especially in cases with lower degrees of VUR. Surgical and endoscopic procedures must be reserved for patients with higher degrees of VUR unresponsive to conservative management or in whom new scars may develop.

Keywords: vesicoureteral reflux, pediatric, urinary tract infection

Introduction

Vesicoureteral reflux (VUR) is one of the most common pediatric urologic abnormalities which affects 1-2% of children [1]. VUR is defined as the retrograde flow of urine from the bladder into the ureters and renal pelvis [2]. It is a congenital anomaly which can result in significant sequels like repeated pyelonephritis and the resultant renal scarring, hypertension and renal insufficiency [3]. It is still one of the most common causes of renal failure in children [4]. VUR is classified as primary or secondary to a concomitant condition [5]. Primary vesicoureteral reflux is the result of anatomical defect of the vesicoureteric junction.
Many advances have been made over the past two decades in understanding the pathophysiology and management of VUR in children.
The aim of the present study was to evaluate the clinical course of children and adolescents with primary vesicoureteral reflux.

Materials and methods

This was a retrospective chart review of 958 children and adolescents 1 day to 14 years old, diagnosed with primary VUR who were admitted to the Pediatric Nephrourology Unit of the Children's Medical Center in Theran, Iran between 1993-2007. This hospital is affiliated with Tehran University of Medical Sciences (TUMS). Patients with secondary VUR and those with incomplete follow-up were excluded from the study.
Patients were diagnosed by voiding cystoureterography (VCUG) in the majority of cases and radionuclide cystography in some patients. VUR grade was classified

Correspondence to: Sepideh Bagheri, Dr Sheikh Children's Hospital, Mashhad, Iran;
Phone: +989 1 55 25 54 51; E-mail: bagheris@mums.ac.ir

according to the International Reflux Study Committee's system [6]. In patients with radionuclide cystography, VUR was graded as mild (corresponding to grade I, II), moderate (corresponding to grade III), and severe (corresponding to grade IV, V).
The following parameters were analyzed in the study: age, sex, clinical presentation, VUR grade, sonographic findings, DMSA changes, treatment modality (medical, surgical or endoscopic) and response to treatment, hypertension (presence/absence), urinary tract infection recurrence and development of new kidney scars in patients under medical treatment.

Statistical analysis

All statistical analyses were performed using SPSS 16 statistical software. The Chi Square test was used for the comparison of proportions.
The study was approved by the Ethics Committee of TUMS.

Results

A total of 958 patients were analyzed in this study. Main baseline data are shown in Table 1.
VUR was bilateral in 310 patients (32.3%). Ultrasound was performed in all patients but it was unable to detect VUR in 548 (57.2%) patients which shows that it is not a reliable modality for detecting VUR in children. This is true especially for lower grade VURs. The prevalence of different grades of VUR in our study population was as follows: I (28.9%), II (24.7%), III (27.3%), IV (9.9%) and V (8.9%).

Table 1. Baseline characteristics of patients (n=958)

	Number (%)
Gender	
Male	441(42%)
Female	607(58%)
Clinical Presentation	
Urinary tract infection	893(86%)
Fetal hydronephrosis	114(11%)
Positive family history	26(2%)
Others	15(1%)
Age	
Mean	2.8
Median	2.1
Range	1 day -14 years

DMSA scan showed renal damage in 41.2% of patients at admission. We found a strong association between severity of VUR and renal damage. Of the 151 patients with severe VUR, 126 (83.4%) showed renal damage on DMSA scan whereas 269 of 556 patients with mild to moderate reflux (48.3%) showed renal damage.
The mean follow-up of patients was 14 months (range 6-36 months). Many patients were lost to follow-up because this was a retrospective chart review and many phone numbers and addresses were changed. Finally 638 patients were followed-up. Of these 386(60.5%) patients were managed medically; 49(7.6%) were submitted to surgical procedures and 203(31.8%) were submitted to endoscopic procedures. Reflux resolution was seen in 63% of patients submitted to medical management. Medical management consisted of prophylactic antibiotic therapy for prevention of urinary tract infection in patients. Reflux resolution was defined as absence of VUR on control VCUG or radionuclide cystography. Reflux resolution with medical management was more signifycant in patients with lower degrees of VUR: 84% in grade I and 78% in grade II. Forty-nine patients were treated surgically of whom 37(67.2%) responded to the surgical treatment.
A total of 203 patients were submitted to endoscopic procedure, of whom 108(53.2%) responded to this mode of treatment and their VUR resolved on follow-up.
Blood pressure was recorded for 834 patients. Fourteen patients (2%) showed blood pressure above the 95^{th} percentile for age, sex and height.
Urinary tract infection during follow-up in patients under conservative management was 11% and 10% in patients submitted to endoscopic management.
At the end of the follow-up period 68(17.6%) of patients submitted to medical management developed new scars on DMSA scans. New scar formation was strongly associated with higher degrees of VUR.

Discussion

In this study we have reported the clinical course of a group of children and adolescents with primary VUR. VUR was slightly more prevalent in girls similar to previous reports [7,8]. Mean age at diagnosis was 2.8 years and was also similar to previous reports [9,10].
41.2% of patients showed renal damage and decreased uptake on DMSA renal scans and this was higher in children with severe degrees of VUR. The association of renal damage with VUR degree has been reported in previous studies, too [8-13].
Our study showed that ultrasound is not a suitable radiologic modality for detecting VUR as it was unable to detect VUR in 57.2% of patients. Elder JS in his study reported the imaging modalities for VUR [14].
The goal of treatment in patients with VUR is to reduce renal parenchymal injury [10]. In our study treatment modalities consisted of medical management, surgery or endoscopic intervention.
Medical therapy is the administration of low-dose antibiotic to prevent urinary tract infection and thus formation of renal scars. Resolution of VUR was observed in 63% of patients who were managed medically. Previous studies have also shown that VUR tends to resolve spontaneously [15,16].
We identified that reflux resolution was more signifycant in patients with lower degrees of VUR. This was also shown in a study by Silva *et al* [10].

Breakthrough UTI was seen with a lower incidence in our study in comparison with previous studies. Perhaps this is due to the retrospective nature of the study. However, the true incidence of UTI could be underestimated. Some studies have reported 57.6% [10], 33% [11], 50% [17] rate of breakthrough UTI in patients with VUR.

Hypertension was observed in 2% of our patients. Reflux nephropathy is one of the leading causes of hypertension in children [18] and studies with longer duration of follow-up have reported a greater prevalence of hypertension among people with VUR.

Smellie *et al.* reported a hypertension prevalence of 6.6% in 226 adults with VUR detected in childhood.[11] Wallace *et al.* followed-up 166 patients with VUR for more than 10 years and found an incidence of hypertension in these patients to be 12.8% [19].

In our study 17.6% of patients developed new scars on DMSA renal scans at the end of the follow-up period, which is similar to previous studies [7,20].

There are some limitations to this study. The most important is the retrospective nature of the study.

But its large sample size and use of different treatment modalities increase the strength of the study.

Conclusion

Ultrasound is not a suitable radiologic modality for detecting VUR in children. Medical treatment by using prophylactic antibiotics alone for prevention of urinary tract infection is effective in many cases of VUR and reflux resolution is detected in many cases under medical management alone. Surgical or endoscopic procedures should be reserved for patients unresponsive to supportive therapy or in whom new scars develop.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 67-72

BJ

BANTAO Journal

Original article

Organ Donation: from Point of View of Students Doing Medical Internship in India

Vaishaly K Bharambe, Vasanti U Arole, Vatsalaswamy Puranam, Purushottam Manvikar and Hetal K Rathod

Patil Medical College, Hospital and Research Center. Pimpri, Pune, India

Abstract

Introduction. To study the knowledge and attitude of a medical student doing internship with regards to organ donation.
Methods. A total of 50 specially designed questionnaires were distributed among medical students doing internship at a medical college. Those who gave their consent to participate in the study were asked to fill out the questionnaire.
Results. 86% gave their consent to participate in the study. 100% were aware of the concept of organ donation. 68% had obtained this knowledge from newspapers. 4% had obtained knowledge from the Medical College. 48%, 48% and 34% believed that an organ donor was live, brain dead and cardiac dead, respectively. Awareness regarding kidney, eye, liver, heart and skin donation was found to be 82%, 80%, 80%, 62% and 64%, respectively. 54% were aware of Law pertaining to organ donation. 90% were either positive or willing to consider organ donation themselves. 10% felt that the donated organ might be misused.
Conclusion. Health care professionals are the first to establish relationship with the potential donor's family and are a crucial link in the organ procurement process. Their attitude and level of knowledge regarding organ donation would reflect directly on the organ donation activity of any region. The interns in the present study had positive attitude towards organ donation but were lacking in knowledge about some key aspects such as brain death and legality involved in organ donation. Majority of the medical professionals had obtained their knowledge from newspapers and very few were taught about organ donation in the medical college. If education on organ donation and its various aspects was included in medical curriculum, it could empower the future medical care professionals with knowledge to further study the cause of organ donation and serve the society better.

Keywords: organ donation, medical interns, kidney transplant, eye donation, brain death

Introduction

All over the world the demand for organs for transplantation exceeds the availability of donated organs [1]. In India, organ donation rate is a dismal 0.34 per million persons per year [2]. Organ donation here is influenced by factors such as education, economic status, religion, superstitions and awareness regarding organ donation.
The attitudes of the health care personnel who closely interact with the family of the deceased potential donor often directly influence the process of organ donation. The low conversion rate of the potential organ donors in India could perhaps be due to the indifferent attitude of some of the health care professionals working in the critical care areas [3].
One of the strategies to increase organ donation rates could be the use of health care professionals for propagating knowledge at the community level [1]. Hence, this study was undertaken to understand the knowledge and attitude of the medical interns regarding organ donation. A discussion session arranged after the research activity also gave opportunity to the Hospital authorities to address any doubts raised by the participating interns.

Objectives

The objectives of the present study included the following:
a) The understand the awareness among medical interns about organ donation.
b) To study their attitude with regards organ donation.
c) To clarify doubts raised by participant interns.
d) To further plan sensitization programs among health care professionals.

Materials and methods

The present study was conducted at our Medical College. All medical interns in one batch (50) were included in the study. A specially designed self-administered ques-

Correspondence to: Vaishaly Bharambe, D 9 State Bank Nagar. Panchvati, Pashan Road, Pune 411008, India; Phone: +982 2 91 08 45; 02 02 58 99 168; Fax: 02 02 78 05 100; E-mail: vaishalybharambe@yahoo.co.in

tionnaire covering demographic data, knowledge and attitude of the interns, was prepared and distributed among the interns.

Inclusion criteria for the study: the participants had to be medical interns who had just passed their final year of undergraduate medical examinations and were serving their internship at our Medical College, Hospital and Research Center. The exclusion criteria: those who refused to participate in the study. The respondents were assured that their confidentiality would be maintained and the study would follow all ethical principles.

The questionnaire was administered after obtaining consent from the participants who were then given 15 minutes to complete the questionnaire. The methodology for filling out the questionnaire was explained to the interns. It was explained to them that the questionnaire was to be filled in privacy without discussing with the other participants. The questionnaires were later collected and data analyzed. This was followed by a discussion session on the topic of organ donation wherein the doubts expressed by the interns were clarified.

Results

Out of the total of 50 medical interns approached for participation in the study, 43(86%) gave their consent to participate in the study.

All respondents were medical interns who had just completed their final year undergraduate medical education in India. They belonged to different states within India and were in the age group between 22 to 26 years old. None of the participants was married. 80% of the participants were followers of Hinduism, and 2% each were followers of Islam and Christianity respectively. About 16% of the respondents were followers of faiths other than those mentioned.

A hundred percent of the participants were aware of organ donation. While 84% were aware of the pressing need to promote organ donation in India, 2% did not believe there was any need to do so and 12% were unsure of the state of organ donation necessity in India.

Newspapers, TV, radio, internet and family members were stated as the sources of knowledge about organ donation by 68%, 16%, 6%, 34% and 18% respondents, respectively. 4% stated medical college education as their source of knowledge.

Regarding categories of organ donors, 48% and 34% believed that an organ donor had to be a living individual or a cardiac dead individual, respectively. Another 48% believed that an organ donor could be a brain-dead individual. Six percent stated that they did know who could be an organ donor.

Investigation into the knowledge of the interns regarding brain death revealed that 58% understood that a person who has been declared as brain-dead has irreversible loss of brain functioning. 12% stated that a brain-dead person was legally dead while 8% stated that the body of a brain-dead person may feel warm. 22% stated that the heart of the brain-dead person would beat. While these 22% were willing to accept the brain-dead person as legally dead, 54% refused to do so and 24% were undecided about whether to consider a brain-dead patient as dead or alive.

Table 1 depicts the awareness of the interns regarding organs that can be donated.

Table 1. Depicts the awareness among the participant interns regarding organs that can be donated in the present study and in studies by another author

	Present study	N F Ali [6], 2013
Kidney	82%	96%
Eye tissue	80%	82%
Liver	80%	72%
Skin	64%	46%
Heart	62%	84%
Lungs	40%	42%
Bone	28%	28%
Heart valves	24%	32%
Pancreas	18%	18%
Entire body	8%	--
Intestine	8%	10%
Ligament	6%	10%

40% of the interns were aware that if the brain-dead donor had not signed a donor card or otherwise expressed his wishes to be a donor, his family members can pledge his/her organs if they believe this could have been his/her wish. 46% of the interns felt that this was not true and 16% were not sure of the answer. 82% of the interns were aware that one single donor could donate to multiple recipients while 18% did not think this as possible.

40% of the interns were willing to be donors, 28% wanted to discuss with their families and 22% were undecided about their willingness to be donors. 58% were willing to sign the donor card. 10% of the interns were clearly unwilling to be donors. 54% and 22% of the interns were positive towards organ donation as it would help others live longer healthier lives and wanted something positive to come out of their death respectively. Eight percent felt that organ donation would help them to continue to live even after their death. 64% of those willing to be organ donors wanted to donate to an unknown individual, 62% to family members, 58% to a friend and 28% to Medical College.

The reasons given by 10% of the interns for unwillinggness to be organ donors are given in Table 2.

Forty percent of the interns believed that committing to organ donation after brain death or cardiac death was every person's social responsibility. 54% were aware of existence of a Law governing all organ donation and transplant activities in India. 52% supported India following opt-out policy of organ donation; 36% themselves were willing to accept an organ donated by a death row prisoner and 30% supported financial aid to family of the donor if they were needy.

Table 2. Depicts the different reasons provided by the participant medical care professionals for refusal to be organ donors (in present study and in studies by other authors)

Present Study	
I fear that the organs may be misused	10%
I do not wish to be cut open or otherwise mutilated	10%
I do not believe in the ability of the system to support the donated organs till they reach a suitable donor	8%
I couldn't be bothered to do all this	6%
I do not believe in organ donation	4%
I live very far away from the closest center of organ donation	2%
It is against my religious beliefs	0%
Bilgel *et al.* [1], 2006	
Organs could be wasted	11.8%
Felt threat to own life (in case of live organ donation)	41.2%
Fear of being cut during surgery (in case of live as well as cadaver organ donation)	17.6%
Lauri and Adami [4], 2010	
Agreeing to organ donation of a family member could be interpreted as showing disrespect to the dead person	
Lack of understanding about the processes involved in organ donation	
Disfigurement of the body of a loved person	
Fear that the brain-dead person may not be actually dead (considering that the heart continues to beat)	

66% of the interns were willing to be a part of organ donation awareness group and promote organ donation activity in society.

Discussion

Organ donation is the most preferred treatment modality for organ failure cases [5]. However, demands for organs for transplantation continues to overwhelmingly exceed the limited supply of organs via organ donation [6]. This extreme shortage of donor organs is especially seen in India where the organ donation rate is a dismal 0.34 persons per million [2].

Research into the poor organ donation rate has revealed one of the causes as lack of awareness among doctors about the criteria for cadaver organ donation and about guidelines for declaration of brain death [5]. The health care professionals occupy a unique position between the lay person and the transplant professional. There is a great need to increase awareness about organ donation among the health care professionals who in turn can then motivate the public and propagate knowledge at the community level. The present study was undertaken with this view in mind. The study explores the attitude and knowledge of young interns, who have just passed their undergraduate medical examinations in India, towards organ donation. A discussion session arranged after the research activity also gave opportunity to the Hospital authorities to address any doubts raised by the participating interns.

Forty-three out of the 50 interns approached, gave their consent to participate in the study (86%). In a study by Chung *et al*. in Hong Kong overall 94% consent to participate in the study was observed, while a 70% response rate by the fourth year medical students was reported by Bardell *et al.* in a study conducted in a medical school in Canada [7,8]. Consent to participate in any study could be considered as indicative of the level of interest in the set topic.

All the participating interns (100%) had awareness about the concept of organ and cadaver donation. Bapat *et al.* also reported that majority of the postgraduate medical students participating in their study had awareness about body and organ donation [5]. 14% of interns were either unaware or unsure of the need to promote organ donation in India. These health care professionals could therefore miss an opportunity to promote organ donation that came their way during their practice. India is severely lacking in organ donors and often missing of an opportunity to convert a donor could mean loss of life to all those who could have received the donated organs such as 2 kidneys, liver, lungs, heart etc.

Table 3. Depicts the sources of knowledge of organ donation as stated by participants in present study and in studies by other authors

	Newspaper	**TV**	**Radio**	**Internet**	**Family members**	**Medical teachers**	**Others**
Present study	68%	16%	6%	34%	18%	4%	
U Bapat *et al.* [5] 2009	60%	61%	31%	-	-	-	26% from magazines, brochures
B Priyadarshini *et al.* [9] 2003	79%			-	-	-	Neighbours, Publicity campaigns
Bilgel *et al*. [1] 2006	72.1%			-	-	22.7%	-
NF Ali *et al*. [6] 2013	42.4%	64.6%		-	50%	27.8%	Neighbours

Table 3 depicts the sources of knowledge of organ donation as stated by participants in the present study compared to other authors. All studies including our study report media as the most important source of knowled-

ge regarding organ donation. Only 4% of the interns in the present study reported medical education as a source of their knowledge. Bardell *et al*. in their study about the knowledge of medical students regarding organ donation found their knowledge to be limited and stated that this could be due to the paucity of teaching on the topic of organ donation in the undergraduate medical curriculum [8]. They stated that there was no formal teaching on aspects such as identification of potential donor or approaching a potential donor family. While it cannot be denied that media is doing a bit to promote organ donation among the general population, organ donation and subsequent transplantation remains a complicated and highly sensitive medical issue. This needs to be taught at medical schools by experienced medical professionals who will give the future doctors an insight into the subject and its social, medical as well as ethical challenges. Chung *et al.* reported low levels of confidence among medical students in Hong Kong while approaching potential donors and their families [7]. Most students believed their medical curriculum was inadequate in providing transplant-related knowledge.

The interns in the present study were aware of live organ donations (48%) as well as donation following cardiac death (34%). Ali *et al.* in a study carried out in Karachi, Pakistan reported that 59.2% of medical professionals participating in the study believed that ideal organ donor was a cardiac-dead person and 36.1% believed it was a living person [6]. 17.7% believed that a donor is a brain-dead person. A comparatively higher, 48%, in present study were aware of brain dead category of donors and 6% were totally unaware of the required health status of a potential donor. While a live organ donor can donate one or two organs and a cardiac dead donor can donate few tissues or body donation for dissection purposes, the brain-dead donor has ability to make a difference in lives of about 50 individuals through the organs and tissues he/she donates. Lack of knowledge about the brain-dead category of donor in a medical health professional will affect the organ donation rate of that nation adversely [10,11].

The knowledge of the interns regarding brain death was further explored in this study. 58% of the interns were aware that brain death meant irreversible loss of brain functioning and 22% accepted that heart would continue to beat, yet only 22% were ready to accept such a heart beating brain-dead person as legally dead. 54% refused to accept a heart beating person as dead. In a study by Sheerani *et al*. involving health care professionals, 44% accepted a brain-dead person with a beating heart was dead and an equal 44% refused to accept this form of death [12]. Bardell *et al.* reported that 64% of the medical students participating in their study believed that the brain-dead patient was dead while 36% were unable to differentiate between brain death and coma [8]. Komolafe *et al.* state that every physician should be able to recognize brain death because patients who are dead should not be treated as though they were alive. This results in loss of precious medical resources and facilities and a missed opportunity of organ donation [13]. A medical care professional who does not accept a brain-dead person as dead would never approach the family of the patient to consider organ donation. Thus, awareness among medical care professionals about brain death and its importance in organ donation activity is essential to raise organ donation rates in any country.

Regarding organs that can be donated, high awareness was observed regarding kidney, eye and liver donations (Table 1). In comparison to our study, higher percentage of the final year medical students was aware about most of the organs being donated in the study by Ali *et al* [6]. In a similar study by Karini *et al.* 26% of the participating medical students were aware of the organs that can be donated [14]. In all, high levels of awareness were noted about commonly donated organs such as kidney, liver, eyes, heart etc. while awareness about other organs and tissue donation was poor. Poor awareness was observed also about body donation. Knowledge about the specific organs and tissues being donated is not only necessary from the point of view of organ donation but also from the point of view of advising further treatment to the patients suffering from organ failure or severe irreversible tissue damage.

Only 40% were aware of the legal right of the family member of the deceased donor to allow organ donation from the donor in case he/she had not signed the donor card or otherwise expressed their wish to be a donor. The rest were either not aware or unsure of the legality in this situation. Bardell *et al*. reported that in all 41% of the participating medical students were aware of the importance of the wishes of the family of the deceased donor in case of potential organ donation [8]. The findings in both studies are similar and indicate necessity to educate health care professionals about legal rights of family of the potential organ donor. If the deceased person had not written or expressed his/her will to be a donor, the person lawfully in possession of the body may allow organ donation unless he believes that the deceased had objection to organ donation [15]. 54% of the interns were aware of the THO Act, which is the national transplant of human organs and tissues act that governs all organ donation activities in India. This awareness needs to be raised to 100%. India has a history of organ trade and "legal transplant activity" here is trying to raise its head through this murky history. Clear ideas regarding legal aspects in the mind of the treating medical professional go a long way in assuring the public that all is above board [16].

90% of the participating interns were positive towards organ donation, out of which 40% were willing to be donors, 22% were unsure and 28% wished to discuss with their family before taking a decision. Only 10% of the participating interns in the present study clearly refused to be organ donors. Despite 90% being positive,

only 58% of participants in the present study were willing to sign the donor card. This is similar to the study by Oluyombo *et al*. where only one out of every 4 persons who were willing to be donors, signed the donor card [17]. Symvoulakis in a study conducted with medical care professionals, reported that 8.7% carried a donor card [18]. Figueroa reported that while 80% of the medical students were willing to be donors, 59% were actually registered and signed the donor card [19]. 49.3% of the doctors participating in the study of Hu D and Huong H showed willingness to be donors themselves [20].

Table 4. Depicts the various reasons cited by participant medical professionals for refusal to be donors in present study and in studies by other authors

Present Study	
Doubt that the system cannot support donated organs	8%
I don't believe in organ donation	4%
I don't want to be cut open	10%
Fear of misuse of organs	10%
No facility for organ donation nearby	2%
Can't be bothered	6%
Symvoulakis *et al*. [18] 2014	
Worried that the donated organs might be misused	53.8%
Hu D and Huang H [20] 2015	
Afraid that organs would be picked up inhumanly and body would be disfigured	49.2%
Oluyombo *et al.* [17] 2016	
Held back by socio-cultural beliefs and traditions	
Fear of mutilation of body after death which is a taboo and against religious belief	
Inexperience and inadequate information	

Table 4 states the various reasons cited by participating medical care professionals for refusal to be donors, in our study and in studies by other authors. Fear of mutilation, disrespectful handling and misuse of donated organs were common reasons cited by the medical care professionals for having reservations about becoming organ donors. Oluyombo *et al.* stated that considering the high percentage of medical care professionals who had reservations about organ donation, it is important to educate them [17].

Table 5. Depicts the motivation behind medical care professionals agreeing to be organ donors in present study compared to another author

Present Study	
It would help others live longer healthier lives	54%
They wanted something positive to come out of their death	22%
Organ donation would help them to continue to live even after their death	8%
Oluyombo *et al*. [17] 2016	
Had permission of their religion to become organ donors	
Knew a previous organ donor	
Had good knowledge about organ donation	

Table 5 depicts the motivation behind medical care professionals who agree to be organ donors in our study compared with another author. It is natural for a medical care professional to wish to help others live longer and healthier lives. However, the effect of the religion is illustrated in the study by Oluyombo wherein mere permission by the religious faith being followed by the medical care professionals encouraged them to want to be donors [17]. Thus, positive effect of a religious faith that is pro-organ donation could be utilized to promote organ donation. Also, "recipients" who benefited by receiving an organ and the "donors" who have carried out the act of organ donation either live or through a family member after brain death are people who can have a powerful effect on the population to promote organ donation. Thus, the points raised by both studies shown in Table 5, can be utilized to encourage more people to become organ donors.

52% of the interns were in favor of India following the opt-out policy of organ donation as is followed by many countries in Europe. Lauri and Adami state that there are 2 main legislations on organ donation, namely opting-in and opting-out [4]. In the opting-in system, organs can be retrieved from the deceased donor, only if permission to organ donation has been given by the family of the donor. The opt-out system is being practiced in countries such as Spain, Austria etc. Here the law allows the doctors to take out organs from the deceased person's body, if they can be used for transplantation purposes without necessarily having to get permission from the family of the deceased donor. Here, a person unwilling to be a donor needs to opt-out of organ donation if he/she does not wish to be an organ donor. The advantage of the opting-out system is that there is no wastage of organs and the number of people on the waiting list is smaller. The disadvantage is that it may be traumatic to the family members who may not be com-

pletely prepared for organ donation. There is also the ethical issue raised by many, especially when the donor is very young, when he/she may not have got the opportunity to opt-out due to sudden trauma resulting in brain death. It is also not prudent to assume that all those who failed to opt-out had no objection to becoming donors, because of apathy or disorganization preventing them from withdrawing [21].

The willingness displayed by the young interns (66%) to promote organ donation activity in the region was heartened and bode well for future of organ donation in this region. However, the present article proves the necessity of arming the medical professionals with correct knowledge as regards organ donation so as to concentrate this positive energy in the right direction, which will result in a raised number of organ donors in India.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 73-76

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Original article

Conversion from Twice-Daily to Once-Daily Tacrolimus Improves Graft Function but has no Influence on Proteinuria in Renal Transplant Recipients

Nikolina Basic-Jukic, Ljubica Bubic-Filipi, Lea Katalinic, and Judita Lelas

Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital centre Zagreb, School of medicine, University of Zagreb and School of medicine, University of Osijek, Croatia

Abstract

Introduction. Tacrolimus extended-release formulation enables once-daily use. Although an increasing number of patients have been converted from twice-daily (Tac-BID) to once-daily (Tac-QD) formulation, the available information regarding the initiation and follow-up of Tac-QD is sparse. In the present study we investigated influence of switch from Tac-BID or cyclosporine to Tac-QD on renal allograf function, proteinuria and protein-creatinine (P/C) ratio.

Methods. Between October 2012 and October 2014, the switch from Tac-BID or cyclosporine to tacrolimus extended-release formulation was done in 129(38% female, mean age 49 years) renal transplant recipients at different time after transplantation. The analysis focused on markers of graft function (GFR, serum creatinine, proteinuria, P/C ratio), liver function (AST, ALT, γGT, alkaline phosphatase) and blood glucose. Clinical data were obtained at baseline (before conversion), 1 month (V1), 6 months (V6) and 12 months (V12) after conversion.

Results. Both serum creatinine and GFR showed a statistically significant improvement. With GFR, signifycant improvement was observed as early as V1 and it continued to increase throughout the study period up to V12 (all between-visit changes were statistically significant). With serum creatinine, mean levels were numerically decreasing throughout the follow-up period, but a significant improvement occurred at V6 and remained significant at V12 (both vs. V0 values). Proteinuria and P/C ratio did not show any significant change through the observation period. In the majority of patients, the baseline values of AST, ALT, GGT, AlP and glucose were within normal limits and did not change significantly through the observation period. Analysis of tacrolimus C0 showed a significant decrease throughout the follow-up period, at practically all visit. This finding was paralleled by a significant tacrolimus dose decrease from baseline to V6 and V12, as well as by a significant decrease of tacrolimus dose/body weight.

Conclusions. Conversion from cyclosporine or Tac-BID to extended-release Tac-QD improves graft function in renal transplant recipients, without influence on proteinuria or P/C ratio.

Keywords: once-daily, twice-daily, tacrolimus, proteinuria, kidney transplant

Introduction

Once-daily tacrolimus (Tac-QD) has the same active component and metabolism as twice-daily tacrolimus (Tac-BID), but is released more distally in the gastrointestinal tract. Pharmacokinetic study performed on 40 kidney transplant recipients converted from Tac-BID to Tac-QD revealed that conversion to the QD formulation decreased intrapatient variability [1]. Additionaly, Tac-QD could offer the potential benefit of improved medication compliance by decreasing pill burden and thereby simplifying dosing schedule. Although an increasing number of patients has been converted from Tac-BID to Tac-QD formulation, the available information regarding the initiation and follow-up of Tac-QD is sparse [2-6]. Observational data are useful adjuncts to randomized, controlled trials, which clarify whether efficacy under controlled conditions translates into effective treatment in everyday practice. Thus, we investigated influence of switch from Tac-BID or cyclosporine to Tac-QD on renal allograft function, proteinuria and protein-creatinine (P/C) ratio in the routine clinical practice.

Materials and methods

The analysis included 129 kidney transplant patients [49 (38%) female] who were at different times after trans

Correspondence to: Nikolina Basic-Jukic, Department of nephrology, arterial hypertension, dialysis and transplantation, University hospital centre Zagreb, Kispaticeva 12, 10000 Zagreb, Croatia; E-mail:nina_basic@net.hr

plantation switched from either regular release of tacrolimus based on individual decision of the attending nephrologist (Prograf® [n=110] or Tacrocel® [n=11]) or cyclosporine (Sandimmune Neoral® (n=8) to prolonged release of tacrolimus (Advagraf®) as part of their immunosuppressive therapy. Switching from Tac-BID to Tac-QD was made on a 1mg: 1mg basis, except in cases of increased trough levels which required dose-reduction.
The analysis aimed to assess the dynamics of markers of graft function (serum creatinine, GFR, proteinuria, P/C ratio), liver function (AST, ALT, GGT, AP) and glucose metabolism (blood glucose) as well as the relationship of these variables with other characteristics/variables (including previous immunosuppressive therapy, underlying disease, tacrolimus C0, mycophenolate mofetil (MM) dose, tacrolimus and MM dose, tacrolimus and steroid dose) at baseline (V0) and at follow-up visits after 1, 6 and 12 months (V1, V6, V12). Study was approved by the Ethics Comitee of the University Hospital Centre Zagreb, and was performed as a part of a project of the Ministry of Sciences.
Friedman's non-parametric one-way analysis of variance with repeated measures was used, with a post-hoc Tukey test to investigate between-visits differences. Spearman rank correlation test was used when appropriate. Testing for potential differences between patient groups was performed using the Kruskal-Wallis one-way analysis of variance with Bonferroni correction for multiple comparisons.

Results

The mean baseline age (at initiation of Tac-QD, V0) (SD; range) was 48.9 (12.6; 19-76) years; the mean age at transplantation was 46.3 (13.4; 14-74) years. The mean duration of dialysis therapy was 54.6 months (54.9 months; 4.1 months-25 years), and the mean time since transplantation to initiation of Tac-QD (i.e. up to V0) was 31.0 months (40.1 months; 13 days-15 years).
A statistically significant serum creatinine level decrease was recorded during the follow-up period ($p< 0.0001$). The post-hoc Tukey test showed significant differences for V0 vs. V6 ($p<0.021$), V0 vs. V12 ($p<0.001$), V1 vs. V12 ($p<0.001$) and V6 vs. V12 ($p<0.001$), while differences for V0 vs. V1 and V1 vs. V6 were not signifycant ($p=0.75$ and $p=0.23$, respectively) (Figure 1). Equally, a statistically significant GFR increase during the follow-up period ($p<0.0001$) (Figure 2) was noted. Significant differences were found for all between-visits differences (except for V0 to V1), with p values from <0.001 to p=0.008.

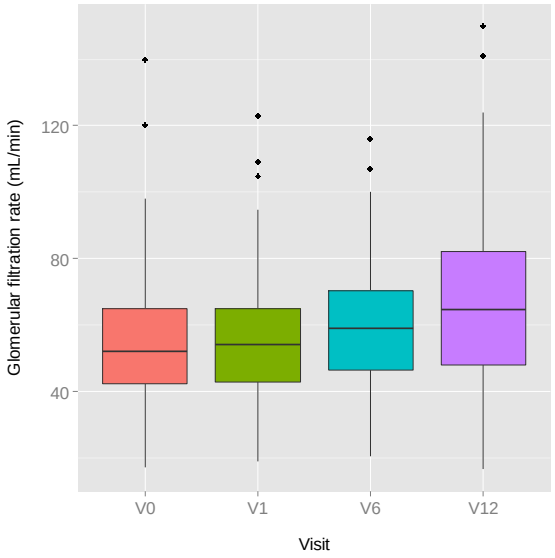

Fig. 2. Glomerular filtration rate increased over the observed period

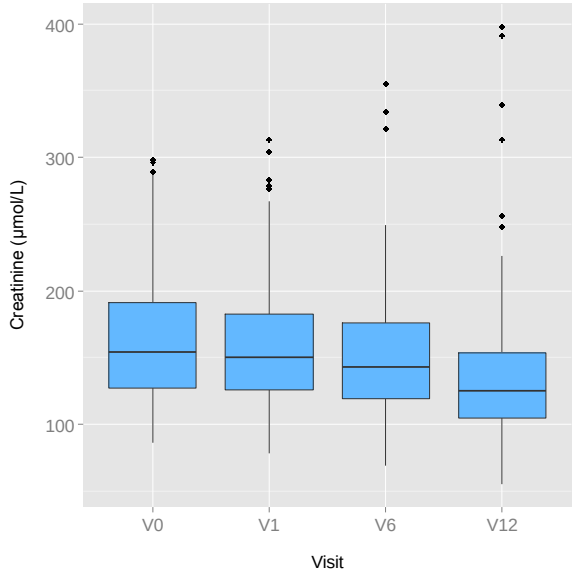

Fig. 1. Serum creatinine decreased during the follow-up. Significant differences were observed for V0 vs. V6 ($p<0.021$), V0 vs. V12 ($p<0.001$), V1 vs. V12 ($p<0.001$) and V6 vs. V12 ($p<0.001$)

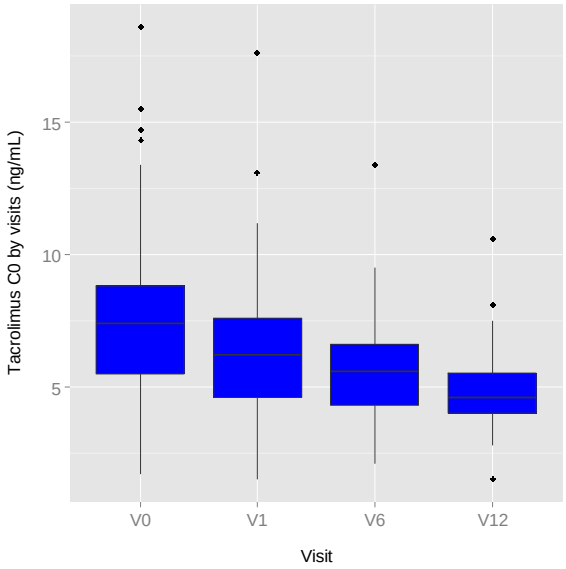

Fig. 3. Tacrolimus C0 trough level decreased throughout the follow-up period

Tacrolimus C0 levels decreased during the follow-up period (p<0.0001). A significant difference was found for V0 vs. V4 (p<0.001), while the between visits p values for V0-V1, V1-V6 and V6-V12 were <0.001, 0.07 and 0.0046, respectively. Significant dose decrease was recorded between V0 and V6 as well as V0 and V12 (both p<0.001) while other pairwise comparisons did not show significant differences (Figure 3).

Upon switching to Tac-QD, tacrolimus dose was decreased in 45 patients, remained unchanged in 59 patients and was increased in 17 patients.

For further analysis, tacrolimus levels were categorized as >5 ng/mL or ≤5 ng/mL; no statistically significant differences were found in GFR at any visit between the patients with lower and patients with higher tacrolimus blood levels (Figure 4).

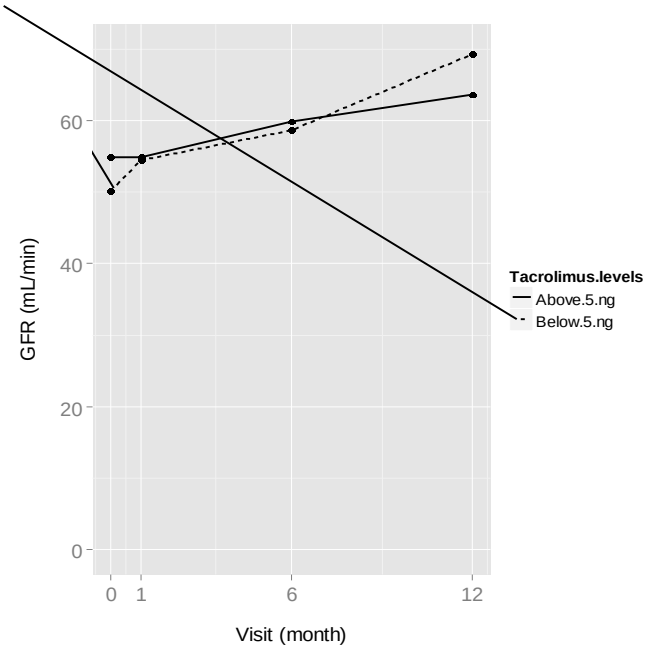

Fig. 4. GFR means grouped by tacrolimus levels (> 5 ng/mL, ≤ 5 ng/mL) through the follow-up period

No statistically significant differences were found for any parameter at any visit between patient groups receiving different mycophenolate mofetil doses.

No statistically significant differences were found between values of individual variables (AST, ALT, GGT, AP) at different visits. Changes in GFR, proteinuria, P/C ratio and enzyme levels (AST, ALT, GGT, AlP) were analyzed with respect to the previously received immunosuppressive therapy (Prograf, n=110; Tacrocel, n=11, Cyclosporine, n=8), with no statistically significant differences found for any variable at any visit.

With the mean follow-up of 2 years (range 12 months to 37 months), among complications recorded after switch to Tac-QD urinary tract infections were recorded in 10 patients, viral infections in 16 patients (1 BKV, 3 CMV, 2 VZV, 10 respiratory viral infections), neurotoxicity in 3 patients (vertigo, dizzines, cognitive impairement), biopsy proven acute rejection in 5 patients, leukopenia in 5 patients, diarrhea in 4 patients, malignancy in 2 patients (planocellular skin cancer, lung cancer) and fungal pneumonia, acute myocardial infarction and stroke in one patient each. New-onset diabetes after transplantation was already present in 13 patients treated with Tac-BID, with stable glycemic control after conversion to Tac-QD.

Discussion

The potential advantages of Tac-QD are better adherence and a safety profile. However, data on outcomes of conversion from Tac-BID to Tac-QD is scarce.

Our study demonstrated the safety and efficacy of conversion from Tac-BID or cyclosporine to Tac-QD at different stages after renal transplantation. We observed a significant reduction in tacrolimus dose in the long term and trough levels accompanied with improvement of renal allograft function. Patients were switched from their previous calcineurin inhibitor (either cyclosporine or tac-BID, Prograf®, Astellas or Tacrocel®, Sandoz) based on individual decision of seven different nephrologists, which is the main value of our observational, non-controlled study, while our data clarify efficacy and safety of Tac-QD in everyday clinical practice.

Hougardy *et al*. retrospectively reviewed data from 55 patients switched from Tac-BID to Tac-QD. They observed a significant increase in tacrolimus daily doses at 6 months (P<0.0001). After conversion, they observed a quick and sustained decrease in trough tacrolimus levels, decreasing from 8.05 ng/mL at day 0 to 6.30 ng/mL at day 180 (P=0.0009). Allograft functions were stable, without episodes of acute rejection [7]. An Italian group

switched 41 patients at 36.6±16.1 months after kidney transplantation from Tac-BID to Tac-QD. All patients maintained stable renal function after the conversion. Adverse events included dizziness and tinnitus in 1 patient, and one acute rejection episode [8]. Nakamura *et al*. switched 33 stable Japanese patients who had undergone kidney transplantation ≥1 years before from Tac-BID to Tac-QD, with the dose conversion ratio 1:1. Patients were followed for 2 months. Graft function remained stable without side effects [9]. Sixty-seven kidney transplant recipients were converted from Tac-BID to Tac-QD. Authors reported that at 2-years postconversion patient survival was 100% and graft survival 98.5%, with low incidence of biopsy-proven acute rejection (6.0%), and favorable safety profile [10]. Additionaly, studies have shown that prolonged release tacrolimus enables steroid withdrawal [11,12].

Our transplant centre prefers low tacrolimus (trough levels around 5 mg/mL) and full recommended dose of mycophenolate. Results of the current study further support this approach with possible additional benefit of Tac-QD instead of Tac-BID. Further follow-up is needed to see possible difference between groups with trough level higher than 5 and lower than 5 ng/mL, while one year after the switch patients with lower tacrolimus trough level had higher GFR (although statistically not significant).

Conclusion

Switch from Tac-BID or cyclosporine to Tac-QD is safe and may improve renal allograft function.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 77-83

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Original article

The Determinants of Hemoglobin Variability in Hemodialysis Patients

Vasilije Tomanoski, Gordana Gjorgjievska, Jasminka Zvezdakovska, Vasiliki Krecova, Aleksandar Andonoski, Margarita Nakovska, Serjat Muharemi, Tode Kliseski, Pavlina Janakievska, Katerina Ristoska, Ramazan Veliu, Liljana Vasileva, Toni Jovanov, Kaltrina Mehmedi-Veseli, Beane Ferati, Jasmina Sterjovska, Sintia Kepeska, and Angela Kachakova

Sistina Nefroplus Centers of Hemodialysis, Skopje, Republic of Macedonia

Abstract

Introduction. Factors that have been reported to affect erythropoietin (EPO) responsiveness in hemodialysis (HD) patients include iron deficiency, chronic inflammation, secondary hyperparathyroidism, malnutrition and inadequate HD dose. The aim of the study was to analyze the deteminants of hemoglobin variability in HD patients.
Methods. The study encompassed 526 patients (197 F and 329 M). According to HD vintage at the beginning of the study the patients were divided into two groups: group-1 encompassed 153 patients with HD vintage bellow 24 months, and group-2 encompassed 329 patients with HD vintage over 24 months. Over a period of 21 months after admission the following parameters were analyzed: hemoglobin (Hb), EPO dose, iron dose, HD dose (eKT/V), transferrin saturation (TSAT), C-reactive protein (CRP), ferritin and serum albumin at 3 months and parathyroid hormone (PTH) at 6 months.
Results. The percentage of patients with Hb>=105g/L significantly improved, and the average Hb level significantly increased in both groups over a period of 21 months. The average EPO and iron dose significantly decreased, but TSAT and ferritin levels significantly increased over a period of 21 months. The average eKT/V and s-albumin values significantly increased, but the average CRP and PTH levels significantly decresead over a period of 21 months. In group-1 EPO dose and CRP, but in group-2 EPO dose, ferritin, HD vintage, and iron dose were statistically significant predictors of the Hb level 9 months after admission.
Conclusions. Insufficient EPO therapy, iron deficiency and chronic inflammation were the main factors of inadequate correction of anemia in HD patients before admission.

Keywords: anemia, chronic kidney disease, erythropoietin, hemodialysis, hemoglobin variability, iron dose

Introduction

The anemia of chronic kidney disease (CKD) is a multifactorial disorder that can be managed successfully by erythropoiesis-stimulating agents (ESAs) administration. The required dose of ESAs is quite variable in different CKD patients or in the same patient at different periods of time. Hemoglobin variability is the fluctuation of hemoglobin above or below the target range over time or the extent to which multiple measured hemoglobin values differ from each other within a given time span, whereas the calculated mean of all hemoglobin levels may still remain within the target range [1,2]. Identification of predictors of hyporesponsiveness to ESAs in hemodialysis (HD) patients may help improve anemia management and reduce hemoglobin variability [3].
Iron deficiency is the most common cause of EPO hyporesponsiveness, underscoring the need for treating pre-existing iron deficiency concurrently with ESA therapy [3,4]. ESAs stimulate bone marrow to accelerate the rate of RBC production. If iron stores are inadequate to meet the demands of ESA-activated bone marrow, the patient will not respond adequately to ESAs.
Besides iron deficiency, various comorbidities contribute to anemia and complicate its diagnosis in patients with CKD. Anemia of chronic disease, often associated with acute or chronic infection, inflammation, malignancy, or autoimmune disease, activates cytokines that impair erythropoiesis [5,6]. Inflammation or infection, often combined with malnutrition and atherosclerosis in MIA syndrome, is considered the most common cause of ESA hyporesponsiveness [6]. Hemodialysis, per se, and the adequacy of hemodialysis dose measured by KT/V have been shown to play an important role in improving anemia and reducing the ESA dosage required for anemia correction in HD patients [7,8]. Hyperparathyroidism is usually listed among the possible reasons for impaired response to ESAs in CKD patients. Possible pathogenic links between anemia and parathyroid hormone (PTH)

Correspondence to: Vasilije Tomanoski, Sistina Nefroplus Centers of Hemodialysis, 1000 Skopje, R. Macedonia; E-mail: tomanoskiv@gmail.com

include reduced erythropoiesis due to calcitriol deficiency, and direct or indirect effects of PTH on erythropoietin release, red blood cell production, survival, and loss [9].
The aim of the study was to determine the prevalence of HD patients with anemia and to analyze the determinants of hemoglobin variability in HD patients.

Materials and methods

The multicenter observational retrospective study encompassed 526 patients (197 F and 329 M) with the average age of 60.98±12.94 years and the average HD vintage of 71.28±69.05 months. According to HD vintage at the beginning of the study the patients were divided into two groups: group-1 (G1) encompassed 153 patients with HD vintage bellow or equal at 24 months and group-2 (G2) encompassed 329 patients with HD vintage over 24 months. Patients' dialysis charts were retrospectively examined to obtain data regarding the erythropoietin (EPO) dose (IU/kg/week) and iron (Fe) dose (i.v. mg/month) in the observed time period. In accordance with ERBP and KDIGO guidelines [10,11] and Health Insurance Fund of Macedonia guidelines for anemia treatment in HD patients the following targets were sustained: hemoglobin 10.5-12.5 g/dl; TSAT: lower limit 20%, target range 30-50%, and serum ferritin: lower limit 100 ng/ml, target range 200-500 ng/ml. Hemoglobin levels were classified as low (<105 g/L), within target (105-125 g/L), or high (>125 g/L). To assess the frequency and the size of the fluctuations in hemoglobin levels over time, we defined six subgroups of patients on the basis of their overall pattern of fluctuation during the first 9 months after admission: consistently low (all 9 months with Hb<105 g/L), consistently within the target range (all 9 months with Hb=105-125 g/L), consistently high (all 9 months with Hb>125 g/L), low-amplitude fluctuation with low Hb levels (LAL- all 9 months with Hb<105 g/L or Hb=105-125 g/L), low-amplitude fluctuation with high Hb levels (LAH- all 9 months with Hb=105-125 g/L or Hb>125 g/L), and high-amplitude fluctuation (HA- within 9 months period with Hb<105 g/L, Hb=105-125 g/L and Hb>125 g/L).
The following laboratory parameters were monitored over the period of 21 months after admission: hemoglobin (Hb-g/L), hemodialysis dose (eKT/V), transferrin saturation (TSAT- %), C-reactive protein (CRP-mg/L), ferritin (ng/ml) and serum albumin (g/L) at 3 months and parathyroid hormone (PTH-pg/ml) at 6 months prior to the mid-week hemodialysis session in the first week of the month. All laboratory values were measured by automated and standardized methods. The continuous variables are presented as the mean values with standard deviation, but categorical variables are presented as percentage. For statistical analysis chi-square test, combined analysis of variance for repeated measures and multivariate regression analysis were performed by the statistical software package SPSS, version 11.5 for Windows.

Results

The prevalent primary renal disease according to the EDTA registry codes in patients were: hypertensive nephropathy in 27.3% of patients, diabetes mellitus type 1 and 2 in 15.5%, glomerulonephitis with no histology in 11%, and Autosomal Dominant/Recessive Polycystic Kidney Disease in 9.1%. Distribution of monthly hemoglobin levels in both groups of patients over the period of 21 months are shown in Figure 1 and 2. The percentages of patients with Hb levels over 105 g/L significantly improved over the period of 21 months in both groups. Nearly 87% of patients in group-1 and 84% in group-2 showed some pattern of Hb level fluctuation during the 9-month study period, as shown in Figure 3. Patients who were classified in the low, target range, and high subgroup in both groups of patients (overall 12.8% in group-1 and 15.66% in group-2) remained stable within their original Hb level ranges during the 9-month study period. Patients who were classified in the

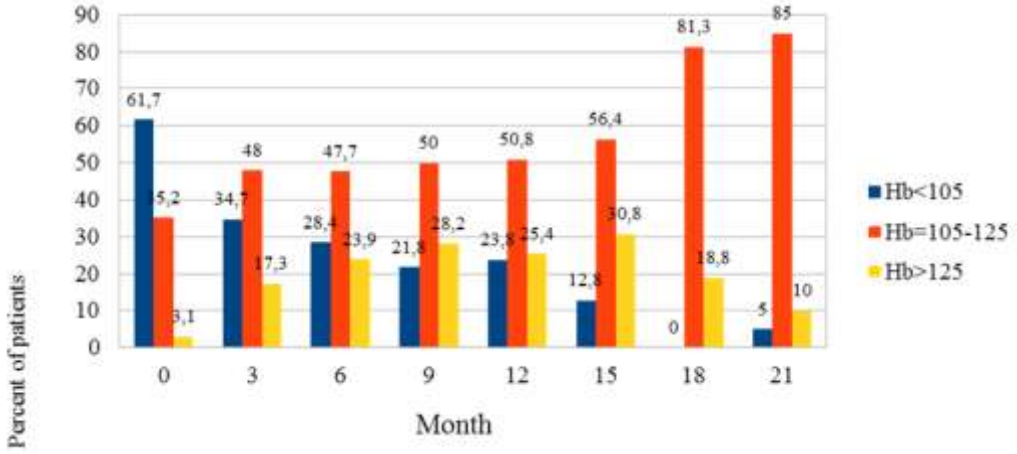

Fig. 1. Distribhution of monthly hemoglobin levels in group-1 of patients

their Hb levels crossed one of the boundaries at 105 or 125 g/L during the 9-month period. Patients who were classified in the HA subgroup showed large fluctuations in their Hb levels, such that they crossed both the upper and the lower boundaries of target range. This was the most common pattern of Hb level fluctuation over time in group-1 (44.48% of patients), with levels falling below or rising above the target range (105-125 g/L) during the 9-month period.

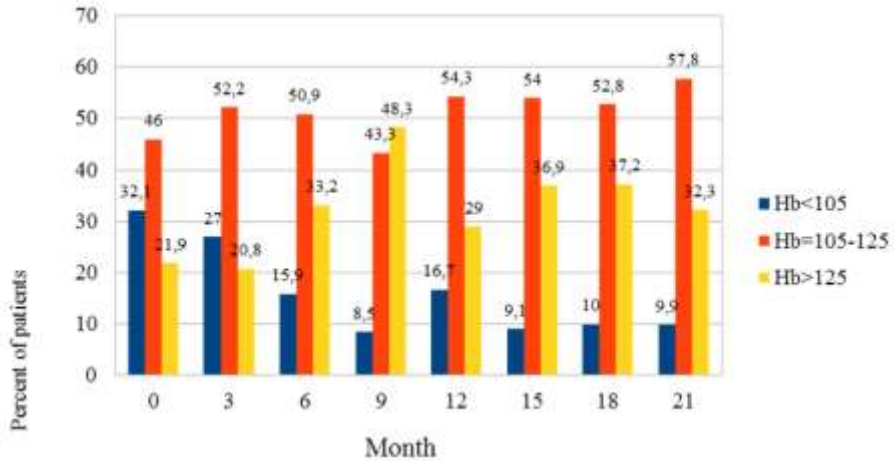

Fig. 2. Distribution of monthly hemoglobin levels in group-2 of patients

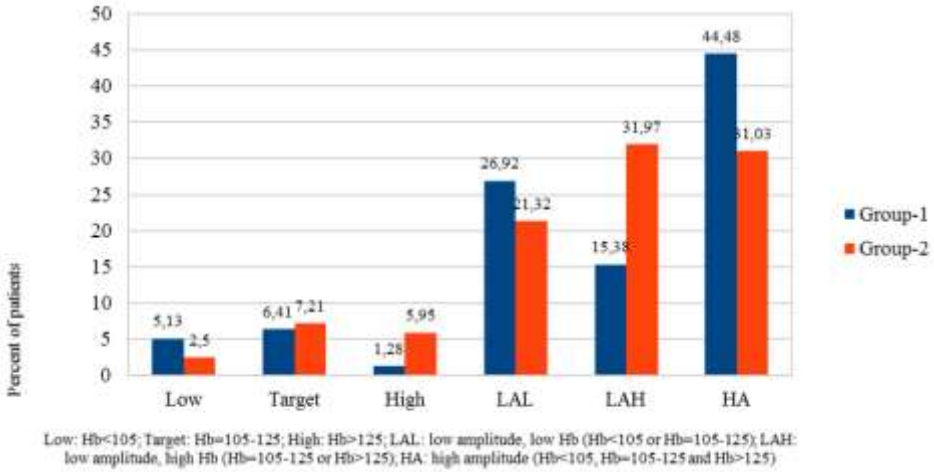

Fig. 3. Patterns of fluctuations in Hb levels during a 9-month period after admission

The average Hb level significantly increased over the period of 21 months in both groups and there was a significant difference in hemoglobin increase between the two groups (Table 1). EPO dose significantly decreased in both groups over the period of 21 months and there was no significant difference in EPO dose decrease between two groups (Table 1). Iron dose significantly decreased over the period of 21 months in both groups and there was a significant difference in iron dose decrease between the two groups (Table 1). The average TSAT and ferritin levels significantly increased over the period of 21 months in both groups and there was a significant difference in TSAT increase, but no in ferritin increase between the two groups (Table 1). The average eKT/V significantly increased over the period of 21 months in both groups and there was a significant difference in eKT/V increase between the two groups (Table 1). The average albumin value significantly increased over the period of 21 months in both groups and there was no significant difference in albumin increase between the two groups (Table 1). The average CRP significantly decresead over the period of 21 months in both groups and there was a significant difference in CRP decrease between the two groups (Table 1). The average PTH significantly decresead over the period of 21 months in both groups and there was no significant difference in PTH decrease between the two groups (Table 1).

In group-1 EPO dose and CRP, but in group-2 EPO dose, ferritin, HD vintage and iron dose were statistically significant predictors of the Hb level 9 months after admission by the model of multivariate regression analysis (Table 2).

Table 1. Mean values of anemia-related factors in both groups of patients over the period of 21 months

Month		0	3	6	9	12	15	18	21	Wilks` Lambda Sig.[a]
Hb (g/L)	G1	98.4±16.4	111.5±15.6	113.0±18.2	115.7±15.8	114.3±17.1	118.2±12.2	119.3±7.3	117.0±8.0	0.736 p<0.001
	G2	111.8±17.0	113.3±15.6	119.5±14.9	124.1±15.2	118.3±15.3	121.4±13.5	121.4±13.8	120.9±13.7	
Sig. between groups		p<0.001	n.s.	p=0.001	p<0.001	n.s.	n.s.	n.s.	n.s.	p<0.001
TSAT (%)	G1	25.4±11.0	27.9±13.7	32.3±15.9	36.9±19.1	36.6±16.7	32.8±15.9	34.7±11.3	34.8±13.0	0.536 p<0.001
	G2	27.3±12.2	30.6±15.6	33.0±17.5	37.0±18.1	36.2±16.5	34.6±15.3	35.5±15.9	37.3±17.0	
Sig. between groups		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	p=0.015
Ferritin (ng/ml)	G1	487±435	512±448	621±485	578±365	640±435	607±436	579±319	588±401	0.863 p<0.001
	G2	416±447	539±494	580±507	573±457	626±450	591±457	640±486	587±448	
Sig. between groups		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
EPO dose (IU/kg/w)	G1	83.7±51.9	87.9±58.5	76.9±61.2	74.1±58.6	66.4±56.2	59.2±51.6	38.2±37.2	36.2±35.6	0.916 p<0.001
	G2	75.0±57.7	71.2±53.6	70.3±60.1	49.8±51.9	50.0±51.1	54.8±54.6	50.4±57.1	44.5±50.5	
Sig. between groups		n.s.	p=0.011	n.s.	p=0.001	p=0.022	n.s.	n.s.	n.s.	n.s.
Iron dose (mg/month)	G1	143.1±117	171.6±128	144.2±129	155.3±126	163.8±137	141.1±120	96.5±105	90.9±110.8	0.969 p<0.05
	G2	110.6±92.7	121.7±109	143.0±113	99.6±109	117±112	96.1±101	78.7±95.4	77.9±96.9	
Sig. between groups		p=0.007	p=0.011	n.s.	p=0.001	p=0.011	p=0.017	n.s.	n.s.	n.s.
eKT/V	G1	1.17±0.33	1.22±0.24	1.28±0.28	1.27±0.24	1.35±0.22	1.36±0.17	1.32±0.20	1.30±0.21	0.855 p<0.001
	G2	1.28±0.30	1.27±0.25	1.35±0.27	1.40±0.23	1.42±0.24	1.42±0.22	1.38±0.22	1.39±0.23	
Sig. between groups		p=0.001	n.s.	p=0.046	p<0.001	p=0.023	n.s.	n.s.	n.s.	p=0.041
Albumin (g/L)	G1	38.5±5.24	40.3±4.73	41.2±4.22	42.4±3.72	41.5±4.27	42.6±3.24	42.6±2.06	43.6±2.60	0.693 p<0.001
	G2	38.4±4.94	40.7±3.86	41.2±4.02	42.7±3.69	41.1±3.70	42.3±3.78	42.2±3.54	43.4±3.74	
Sig. between groups		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
CRP (mg/L)	G1	17.5±32.7	13.7±28.4	11.0±18.4	12.3±25.7	14.4±31.3	10.1±20.4	9.04±17.5	10.0±15.7	0.934 p=0.002
	G2	12.0±15.7	8.48±17.9	8.09±17.7	8.82±14.5	9.01±17.9	7.67±13.9	7.57±12.4	8.46±26.3	
Sig. between groups		p=0.034	p=0.027	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	p<0.001
PTH (pg/ml)	G1		148±142		149±203		155±153		143±100	0.934 p=0.002
	G2		433±558		304±355		281±337		298±345	
Sig. between groups			p<0.001		p<0.001		p=0.021		p=0.029	n.s.

[a] Combined analysis of variance for repeated measures; G1-group-1; G2-group-2

Table 2. Predictors of hemoglobin variability in both groups of patients 9 months after admission by multivariate regression analysis

	Beta	Significance	Part
EPO	G1: - 0.377	p=0.031	- 0.283
	G2: - 0.376	p<0.0005	- 0.360
Iron dose	G1: 0.010	n.s.	0.008
	G2: - 0.119	p=0.037	- 0.114
Ferritin	G1: - 0.085	n.s.	- 0.075
	G2: - 0.215	p<0.0005	- 0.210
CRP	G1: - 0.345	p=0.030	- 0.286
	G2: - 0.106	n.s.	- 0.100
HD vintage	G1: 0.035	n.s.	0.033
	G2: 0.164	p=0.006	0.149

Model Summary: Dependent Variable Hb- 09th month, Group-1 (G1): ANOVA: F=3.057; R Square= 0.44; Sig. p = 0.008, Group-2 (G2): ANOVA: F=11.904; R Square= 0.316; Sig. p< 0.0005

Discussion

The most noteworthy aspect of intrapatient variability in Hb levels in HD patients is its common occurrence. Minor fluctuations above and below the target range may be normal in any setting. However, wide and prolonged fluctuations in Hb are usually associated with several internal and external factors that can influence ESA response and Hb stability. One study found that hemoglobin cycling more than 1.5 g/dL above or below the target range and lasting more than 8 weeks occurred in more than 90% of 281 HD patients, and that the mean number of hemoglobin excursions per patient was 3.1 per year, with a mean per-excursion amplitude of 2.5 g/dL and a mean duration of 10.3 weeks [12]. Among our study population, nearly 87% of patients in group-1 and 84% in group-2 showed some pattern of Hb level fluctuation over time, which is in accordance with findings of other studies [1,12]. The significant proportion of the patients in group-1 and much less in group-2 had Hb below target of 105 g/L at admission. The EPO doses were significantly reduced over the 21-month period after admission in both groups of patients as the average Hb level significantly increased in both groups. The hemoglobin increase was significantly faster and higher in group-1 of patients in comparison with group-2. Adjusting epoetin doses when patients' Hb levels exceed the upper level of the target range of 125 g/L may be a major source of the fluctuation of patients' levels once they reach that point. When evaluating the relation between ESA and hemoglobin levels cross-sectionally within the population, a higher ESA dose is associated with a lower hemoglobin level as a result of the "confounding by medical indication". This phenomenon, has been previously described for the administered doses of ESAs in CKD populations [18]. HD patients with ESA-hyporesponsive anemia paradoxically receive *higher* ESA doses to achieve the same hemoglobin target as those who are better responsive.

Besides EPO deficiency, the most common additional cause of anemia in CKD is iron deficiency [6]. Iron deficiency can be either absolute, whereby iron stores are depleted, or relative, whereby iron stores are replete but circulating iron is deficient. Relative iron deficiency is also known as *functional iron deficiency* because functionally available (transferrin-bound) iron is insufficient to meet physiological needs. The peripheral iron markers serum ferritin and TSAT are most commonly used as the basis for treatment decisions in CKD patients. In iron-deficient erythropoiesis, declining TSAT occurs in the presence of normal serum ferritin levels [14]. The KDOQI guidelines recommend iron supplementation as an adjuvant to ESA treatment of hemodialysis patients in order to maintain serum ferritin greater than 200 ng/mL and TSAT greater than 20% and to minimize the ESA dosage needed to achieve the target Hb range [15]. However, in view of the divergent TSAT and serum ferritin values TSAT is considered to have greater relevance as a predictor of positive response to iron therapy [14]. Intestinal absorption of oral iron is low and the clinical response is relatively slow. Intravenous iron readily corrects iron deficiency and avoids two limitations of oral iron formulations: impaired absorption and gastrointestinal side effects [16]. In our study population iron deficiency was significantly more frequent in group 1 of patients at admission and greater iron doses in the first 2 months after admission were administered in comparison with group 2 of patients. Therafter, the iron doses were significantly reduced over the 21-month period in both groups of patients as the mean TSAT and ferritin levels in both groups of our patients increased. There was a positive correlation between EPO dose and iron dose in both groups of patients. In group 1 of patients iron dose negatively correlated with ferritin level and in group 2 hemoglobin level negatively correlated with iron dose that confirms the above-mentioned phenomenon of "confounding by medical indication" [13].

Inflammation and oxidative stress are typically present in individuals with CKD [17]. Thus, responsiveness to endogenous EPO or ESA therapy may be impaired by anemia of chronic inflammation. The chronic inflammatory state of HD produces a state of functional iron deficiency due to reticulo-endothelial blockade requiring a much higher than normal serum ferritin to provide

adequate iron delivery to the marrow. Inflammation due to chronic comorbidities or intercurrent events, including hospitalization and hemodialysis itself, is now recognized as a major contributor to CKD-associated anemia and ESA hyporesponsiveness that may require administration of higher ESA doses [18,19]. In our study population the mean CRP level was significantly higher in group 1 of patients in comparison with group 2 of patients in the first 2 months after admission. The mean CRP levels in both groups of our patients significantly decreased over the period of 21 months. In both groups of patients there was a significant negative correlation between hemoglobin level and CRP, as well as hemoglobin and ferritin level, and positive between EPO dose and CRP, but in group 1 of patients there was a positive correlation between EPO dose and ferritin. These observations confirm the findings in other studies [18,19] that chronic inflammation worsens anemia in hemodialysis patients and higher EPO doses are required.

A large proportion of HD patients have also protein energy malnutrition and wasting, low serum albumin levels and a diminished nutritional status could be a feature of patients who are resistant to ESA treatment [20]. In our study population the mean albumin levels in both groups of our patients significantly increased over the period of 21 months. In group 1 of patients there was a significant positive correlation between hemoglobin and albumin level and negative between albumin and EPO dose, but in both groups there was a significant negative correlation between albumin and CRP that confirm the findings of other studies [19-21] that MIA syndrome worsens anemia in hemodialysis patients and higher EPO doses are required. In both groups of our patients there was a significant negative correlation between albumin and age that confirm the findings in another study that malnutrition is more common in elderly than in younger patients [21].

The benefit of adequate HD dose measured by KT/V in improving anemia and reducing the ESA dose required for anemia correction in patients with ESRD [7,8] may be due to the correction of oxidative stress, and the removal of molecules that inhibit erythropoiesis. In our study population the mean eKT/V value was significantly higher in group-2 of patients in comparison with group-1 in the first 12 months after admission. The mean eKT/V value in both groups of our patients significantly increased over the period of 21 months. In group-1 of patients there was a significant positive correlation between hemoglobin level and eKT/V and in group-2 there was a significant positive correlation beween eKT/V and HD vintage, and eKT/V and ferritin. These observations are in accordance with findings in other studies [7,8].

Hyperparathyroidism may directly cause ESA-hyporesponsiveness by diminishing endogenous erythropoietin synthesis, reducing bone marrow erythroid progenitors, and shortening erythrocyte survival [22]. Indirect effects include the association of renal osteodystrophy with bone marrow fibrosis [23], confirmed by the observation of the restored bone marrow space and concomitant rise of serum erythropoietin concentrations after parathyroidectomy [24]. In our study population the mean PTH level was significantly higher in group 2 of patients compared to group 1 of patients over the period of 21 months. The mean PTH levels in both groups of our patients significantly decreased over the period of 21 months. In both groups of patients there was a significant negative correlation between PTH and age that confirms the findings in another study that adynamic bone disease with low PTH level is prevalent in elderly HD population [25]. In group 2 of patients there was a significant positive correlation between PTH and HD vintage that confirms the findings of other studies [22, 26] that secondary hyperparathyroidism progresses in hemodialysis patients if inadequatly managed.

Limitations of the study

The main limitations of this study are its observational and retrospective nature and missing hospitalization and outcome data. Because of the retrospective nature of the study, our results should be interpreted as associative rather than causative. The hospitalization rate and the ESA dose during hospitalization were not accounted for and therefore we cannot determine its effect on our data and the resulting error term of the model may be increased. Another limitation of our study is that it is based on only 21-month follow-up period of the cohort, rather than a longitudinal one of several years.

Conclusions

Insufficient EPO therapy, iron deficiency and chronic inflammation with protein-energy wasting were the main factors of inadequate correction of anemia in patients on maintenance hemodialysis before admission, especially in the group of patients with hemodialysis vintage below 24 months.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 84-88

BJ

BANTAO Journal

Original article

Are Depression and Anxiety Common in Hemodialyzed Patients?

Nada Pop-Jordanova and Momir Polenakovic

Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

Abstract

Researchers confirmed that depression and anxiety are two common comorbid disorders in chronic kidney patients.
The aim of our study was to screen the level of depression and anxiety in a group of end-stage kidney diseases treated with hemodialysis.
The evaluated sample comprised 230 participants; 110 females (mean age 55.5±13.5 years), and 120 males (mean age 54.5±14.3 years). The mean duration of maintenance dialysis was 8.3±5.8 years (from 0.5 to 24 years). Patients were selected randomly from three dialysis centers in R. Macedonia.
As psychometric instruments Beck Depression Inventory (BDI) and scores from Minnesota Multiphasic Personality Inventory (MMPI-201) were used.
Our study confirmed that majority of evaluated dialyzed patients are depressed and anxious in different level, but unfortunately the mental problems are frequently unrecognized.
We suggested some response measures for management of these conditions in order to avoid risks for complications as well of suicide.

Keywords: dialysis, depression, anxiety, management

Introduction

Depression has been called "the common cold" of mental health because epidemiological data shows an enormous percentage of affected population worldwide.
In the report about global World Health in 2008 it was pointed that depression has a greater effect on overall health than angina, arthritis, asthma or diabetes. The World Health Organization (WHO) has ranked depression the 4th leading cause of disability and projects that by 2020, it will be the second leading cause [28,24-27].
The WHO World Mental Health (WMH) Survey Initiative estimate in evaluated 18 countries the percentage of depression in general population to be ranged from 2.2% (Japan) to 10.4% (Brazil) [26].
Depression can have many causes. In the case of someone who has just been diagnosed with chronic kidney disease there may be a lot of information to process about the physical health, which could lead to strong emotions about the life and how it may change. Similarly, once a person reaches end-stage renal disease and begins dialysis, there are lifestyle adjustments to be made that could bring up feelings of despair. Many times these feelings are only temporary, but depression can also be persistent and severe. The core rule worldwide is that when depression is diagnosed in any person, especially in chronic ill one, the psychiatric/psychological help is inevitable. On the other side, just because someone has kidney disease, or end-stage renal failure, doesn't mean he will obligatory experience depression [2,3,6- 9,13].
Searching for depression in end-stage renal diseases, only in Medline we could find more than 1400 articles devoted to this issue, especially when the hemodialysis is used as maintenance therapy.
Depression can be described as being composed of two components: an affective (mood) one and a physical (somatic) one (e.g., loss of appetite, fatigue). In participants with concomitant physical illness like chronic renal failure, the reliance on physical symptoms may artificially inflate scores obtained on psychometric questionnaires due to symptoms of illness, rather than those of depression. For this reason, there are several precautions that must be taken into account when interpreting the results.
The gold standard for diagnosis depression are clinical signs described in DSM-V and scores obtained with psychometric instruments. In the DSM-V, depressive disorders are classified in three subtypes: major depression, persistent depressive disorder, depressive disorder NOS (not otherwise specified), while minor depression is classified in the group of NOS depressive disorder. Symptoms of depression include feelings of worthlessness or guilt, diminished ability to think, concentrate or make decisions, weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, and recurrent suicidal ideation. As psychometric instruments the most frequently used are: Hamilton scale (HAM-D), Zung Self-Rating Depression Scale, Montgomery-Asberg Depression Rating Scale, Geriatric Depression Scale, Beck Depression Inventory etc. Except the HAM-D (used by professionals), which was developed as a measure of treat-

Correspondence to: Pop-Jordanova Nada, Macedonian Academy of Sciences and Arts, Bul. Krste Misirkov 2, 1000 Skopje, R. Macedonia; E-mail: popjordanova.nadica@gmail.com

ment outcome rather than a screening or diagnostic tool for depression, all others are based on self-reports [10,17,18].
The most frequently used self-report measures for depression are Beck Depression Inventory I and II, Center for Epidemiologic Studies Depression Scale, Geriatric Depression Scale, Hospital Anxiety and Depression Scale, and Patient Health Questionnaire-9. Some of these measures have become integrated into routine clinical practice (as screening tools) in large managed-care organizations.
Anxiety is characterized by many symptoms such as: feelings of panic, fear, and uneasiness, problems sleeping, cold or sweaty hands or feet, shortness of breath, heart palpitations, not being able to be still and calm, dry mouth, numbness or tingling in the hands or feet, nausea, muscle tension, dizziness etc. There are several forms of anxiety: panic attack, social phobia, generalized anxiety disorder or specific phobias.
Anxiety disorders may be caused by problems in the functioning of brain circuits that regulate fear and other emotions, but the exact cause of anxiety is still not known. Certain environmental factors, such as a trauma or significant event, may trigger an anxiety disorder in people who have an inherited susceptibility to developing the disorder. Anxiety disorders are highly prevalent in the general population, comprising 7-8% of patients seen in a primary care settings, or approximately 40 million adult only in USA. There are significant overlap in symptomatology between depression and anxiety disorders and these two conditions often simultaneously coexist in hemodialyzed patients [6,9,28].
Anxiety and depression may complicate the treatment of any medical condition, especially the chronic one. There might be direct effects, where anxiety and depression have adverse physiological manifestations, but also some indirect effects, particularly behavior problems which may disturb relationships with the environment of patients.
The aim of this article is to present our finding concerned to depression and anxiety in patients on maintenance hemodialysis.

Materials and methods

In this research the original BDI was used for screening depressive symptoms in dialyzed patients. The Beck Depression Inventory follows the criteria for depression listed in the DSM-IV. The test consists of twenty-one questions that assess the not only presence of depression, but also the severity of depression as well. The BDI is widely used as an assessment tool by health care professionals and researchers in a variety of settings. Each question has a set of at least four possible responses, ranging in intensity. When the test is scored, a value of 0 to 3 is assigned for each answer and then the total score is compared to a key to determine the depression's severity. The standard cut-off scores are as follows: 0-9: indicates no/minimal depression; 10-18: indicates mild depression; 19-29: indicates moderate depression; 30-63: indicates severe depression [4]. Some researchers suggested the higher cutoff score of BDI (over 14) when chronic patients are tested with. A BDI cutoff of 14 had a sensitivity of 62% and a specificity of 81% for identifying depressive disorder [10]. This was attributed to the overlap between somatic symptoms of depression and symptoms related to the end-stage renal illness, like anemia, fatigue, difficulty concentrating, difficulty sleeping, and poor appetite.
The level of anxiety in this sample is calculated indirectly, using scores obtained in MMPI-201. [in 37]. The anxiety index is calculated using the formula:

$$\mathbf{AI = 1.33D + 1.00\ Pt - 0.66Hs - 0.66Hy}].$$

As evaluated sample, patients treated with chronic maintenance dialysis comprised 230 participants; 110 females (mean age 55.5±13.5 years), and 120 males (mean age 54.5±14.3 years). The mean duration of maintenance dialysis was 8.3±5.8 years (from 0.5 to 24 years). Patients were selected randomly from three dialysis centers in R. Macedonia.

Results

Results obtained with the Beck Depression Inventory are presented in Table 1.
The incidence of depression in the general population in the Republic of Macedonia is 5.2%. The calculated difference between the percentage of depression in dialyzed patients (total 67.84%) versus depression in the general population (5.2%), shows high statistical significance ($p<0.01$). It is obvious that depression is much more expressed in hemodialysis patients than in the general population.

Table 1. Depression in dialyzed patients

Level	Percent
severe	14.28%
moderate	17.85%
mild	35.71%
minimal	21.43%

If we use higher cutoff, minimal depression will be transformed in a group without any depression (21.43%) because obtained scores were below 10. Still, 67, 84% of all dialyzed patients confirmed some level of depression.
It was interesting to see the influence of age and education on depression level. With this purpose we calculated the Pearson's correlation between the age of patients and depression scores (Figure 1). The data we obtained confirm that age significantly influences the development of depression (correlation $r=0.33$; $p=0.02$). It is well known that bad mood, sadness, helplessness, and fatigue, which are the most significant signs of depression, are much more expressed by older persons.

The education of patients was as follows: university level -11,3%; high school-60,4%; elementary school-28,3%. Concerning educational level and depression, the obtained calculation shows a small negative correlation which is not statistically significant (r=-0.029; p=0.072) (Figure 2). However, we suppose that higher education could help to find easier the successful coping mechanisms.
In general, this study confirmed that patients on dialysis manifest different levels of depression, implicating the need for some response measures.

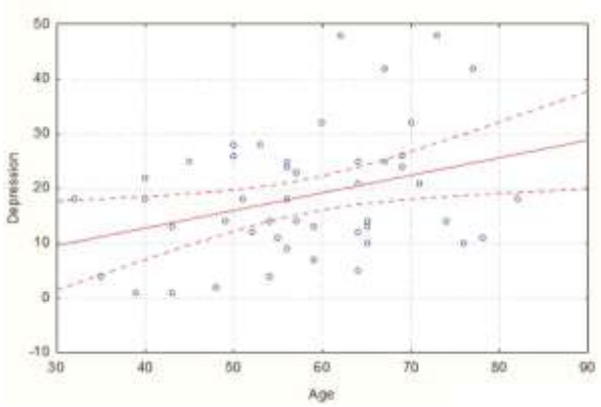

Fig. 1. Correlation between age and depression

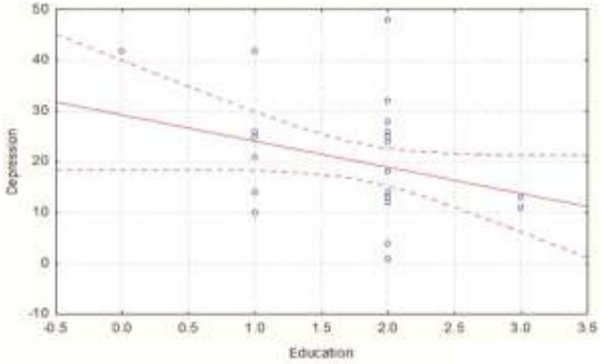

Fig. 2. Correlation between depression and educational level

The selected sample of dialyzed patients was evaluated with MMPI-201 for analyze the personality profile in patients. As an instrument, MMPI-201 allow not only the analysis of personality profiles but also it give the possibility to calculate some additional indexes for the assessment of control mechanisms, aggressiveness, anxiety, or psychosomatics. The anxiety index is calculated using the formula: AI=1.33D+1.00 Pt- 0.66Hs-0.66Hy.
We obtained relatively high anxiety index: AI=27.55.
In our sample of dialyzed patients, the obtained internalization ratio (IR=1.47) shows that patients on maintenance dialysis have relatively good emotional control. Similarly, the expressive-repressive index is quite satisfactory (ER=13.93). It means that the patients can establish a balance between different emotional states. The index of frustration tolerance is relatively high (FT=0.58) which means that patients can cope with many frustrations related to the dialysis treatment as well as with everyday life.
Two other indexes calculated from MMPI scores are: active hostility (AHI=6.08); and passive aggression (PAI =80.1). The results obtained could be related to suppressed hostility, but also to the high passive aggression in these patients. High anxiety is suppressed and cognitively elaborated, reducing active hostility, but it provokes a passive aggression as a general emotional characteristic. The life experience obtained through the aging process as well as long-term experience as chronic patients could be the reason for these personality characteristics.
Obtained results confirmed that dialyzed patients are depressed and anxious, and these two comorbid disorders could influence the long-term prognosis and quality of life.

Discussion

It was pointed in many studies that depression is highly prevalent and associated with poor quality of life and increased mortality in patients with chronic kidney disease, especially those on hemodialysis or those expecting transplantation. Depression and anxiety were attributed to psychosocial and biological changes that accompany dialysis. Additionally, depression has been associated with poor quality of life and adverse medical outcomes in patients with chronic kidney diseases [2,5,6,19-23,42].
Generally, two potential strategies for screening depression in patients with end-stage renal disease are used: the first is conservative and comprises to screen only patients with clinical signs of depression; the second is to screen all new patients periodically, every 6 months to one year with questionnaires. The second strategy seems us to be better.
Murtagh *et al*. in 2008 [32] estimated the prevalence of anxiety in hemodialyzed patients to be 12-52% which depends on different screening methods for anxiety. Cucor *et al.* [6-8] found that 45.7% of selected patients on hemodialysis met criteria for anxiety disorder.
Prevalence of depression in the study of Paimer *et al*. (2013) [34] was calculated to be 39.3% when evaluated by screening questionnaires, and 22.8% when evaluated by clinical interview.
Fischer *et al*. (2011) [14] in a largest study to date reported prevalence of depression of 27.4% using BDI cutoff of 11. Our study showed higher prevalence of depression in evaluated group.
It is supposed that depression is highly correlated with diabetes mellitus. Having in mind that diabetes is closely related to end-stage renal diseases, it seems us logical that these patients manifest depression as a comorbid disease.
Depression and anxiety in end-stage renal diseases have been significantly associated with adverse medical outcome, including emergency visits, hospitalizations, cardiovascular complications, peritonitis, withdrawal from dialysis and suicide. In some studies depression was related to younger age of patients, female gender, and duration

of dialysis, cerebrovascular diseases and diabetes. In our study, age is negatively correlated to the level of depression which means that older patients are more depressed that the younger ones. Lower socioeconomic status have also been associated with higher prevalence of depression.

The cause of depression in end-stage renal diseases, especially dialyzed ones is not clear. Some authors suggest that the mechanisms of depression are similar to those in other chronic diseases. Kutner *et al.* (1985) [28] believed that dialysis patients' depression and anxiety levels are closely tied to their physiological status physiological status.

Kimmel *et al.* (2005) [22] found an increased level of depressive affect correlated with both laboratory and behavioral markers of poor compliance. Decreased behavioral compliance with the dialysis prescription correlated with an increased level of depressive affect in prevalent hemodialysed patients.

Frequent clinical or hospital visits, dietary regime, use of many medications, regular blood pressure control, glucose control and weight may lead to depression. The three time a week hemodialysis procedure is additional challenge. Comorbid conditions such as cognitive decline, stroke, and heart failure are additional risks for depression.

Kurrela *et al.* (2005) [29] pointed that persons with end-stage renal diseases significantly more commit suicide than persons in the general population general population. In a study of Shiraziar S. *et al.* in 2017 [43] it was accentuated that there is significant difference between patients with chronic renal diseases and end-stage kidney diseases related to mental problems.

The antidepressive drugs used in dialyzed patients must be prescribed with special precaution. Opposite, some other non-pharmaceutical approaches are available, but still not used frequently such as: biofeedback, neurofeedback, cranial electrostimulation, different relax training techniques, music therapy, exercise training etc. Cognitive-behavioral therapy is also useful. The choice of therapeutic method depends on the doctors [30,33,35-39, 44]. However, in our centers for dialysis medical staff is not enough educated to screen patients with anxiety and depression, so these two conditions are frequently unrecognized [40].

Conclusions

The study confirmed that anxiety and depression are common comorbid conditions in end-stage renal diseases, especially in patients on maintenance hemodialysis.

We showed that depression is related to age, but not significantly with the educational level or with the duration of dialysis.

It was pointed that these two conditions must be screened regularly in all patients and some response measure must be done.

Education for all staff in centers for dialysis must be organized in order to help the psychological problems related to quality of life and preventing worsening of the outcome.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 89-91

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Case report

A Case of Multiple Myeloma Diagnosed by Renal Biopsy

Lutfiye Bilge Caliskan[1], Tugba Karadeniz[2], Sumeyye Ekmekci[2], Banu Yilmaz Avcioglu[3], Mehmet Tanrisev[3], Muhammed Ali Kaypak[1], Hakan Yarkici[1], Cengiz Ceylan[4] and Harun Akar[1]

[1]Department of Internal Medicine, [2]Department of Pathology, [3] Department of Nephrology, [4]Department of Hematology, Tepecik Educational and Research Hospital, Izmir, Turkey

Abstract

Multiple myeloma is a malignant disease that results in the proliferation of a single plasma cell clone. The clinical manifestations are anemia, bone pain, bone fractures, hypercalcemia, hypergammaglobulinemia, increased erythrocyte sedimentation rate, rouleaux formation on the peripheral blood smear and rarely increased serum viscosity. Rarely cast nephropathy associated with acute renal failure may be the first finding of multiple myeloma. We report a clinical case of a 44-year-old female patient who presented with acute renal failure due to cast nephropathy without myeloma's typical clinical and laboratory findings. In the clinical case presented here, we highlight that multiple myeloma can be presented with acute renal failure and without any other typical symptoms.

Keywords: Multiple myeloma, renal biopsy

Introduction

Plasma cell dyscrasias result from a clonal expansion of neoplastic plasma cell. In general, plasma cell dyscrasia can be detected by the presence of one of the following findings: monoclonal light chain in the serum by immunofixation electrophoresis (SIFE), monoclonal light chain in the urine by immunofixation electrophoresis (UIFE), or monoclonal plasma cells in the bone marrow by immunohistochemistry [1]. The diagnosis of plasma cell dyscrasias including multiple myeloma (MM) can be done by bone marrow aspiration, biopsy and clinical laboratory test [2]. The diagnosis of MM requires assessment of a Wright-Giemsa stained bone marrow aspirate and a hematoxylin and eosin stained core biopsy section [2]. Kidney injury represents one of the leading characteristics of plasma cell disorders [3] and kidneys are target organs in plasma cell disorders [4]. In other words, renal function is often impaired in plasma cell disorders and this is due to the presence of monoclonal proteins [4]. Myeloma cast nephropathy is one of the most common types of kidney injury [3]. The term myeloma kidney or myeloma cast nephropathy generally refers to renal insufficiency caused by the tubulointerstitial damage. Myeloma-induced renal failure is associated with significant morbidity and mortality. Rapid intervention is critical in order to reverse kidney damage and improve renal function. In addition to clinical suspicion, further evaluation is often necessary.

Case Report

A 44-year-old woman with absent previous medical history came to the outpatient clinic complaining of nausea, vomiting and weakness over a period of 2-weeks. The patient was admitted to the Department of Nephrology for evaluation. She reported no back pain and no medication known to be associated with renal dysfunction. The patient's vital signs and physical examination were normal. Cardiac and pulmonary examination showed no abnormal findings. Routine laboratory tests were as follows: Hgb: 9.9 gr/dl, Hct: 29%, MCV: 89.5 fl, PLT: 187.000 u/l, WBC: 10.900 u/l, glucose: 98 mg/dl, urea: 157 mg/dl, creatinine: 7.3 mg/dl, potassium: 5.5 mEq, AST: 16 U/L, ALT: 24 U/L, protein: 8 g/dL, calcium: 10.4 mg/dL, phosphorus: 5.1 mg/dL, uric acid: 4.9 mg/dl, albumin: 4g/dl, globulin: 4g/dl, T. bilirubin: 0.5 mg/dl, erythrocyte sedimentation rate: 39 mm/h. Urine examination was unremarkable. Review of the peripheral smear demonsrated anisocytosis. Iron parameters and LDH were within normal limits. An abdominal ultrasound scan demonstrated normal-sized kidneys with increased echogenicity without evidence of obstruction. At the time of the evaluation, she was treated appropriately with hemodialysis. The patient underwent renal biopsy in order to elucidate the etiology of the acute renal failure. Subsequent pathology report revealed cast nephropathy. No lytic lesions were detected on direct radiographs. Monoclonal lambda-free light chain was found in serum and urine immunofixation electrophoresis, and serum immunoglobulin levels were as follows: IgA: 173 mg/dl, IgG: 1108 mg/dl, IgM: 113 mg/dl. Bone marrow aspiration revealed 35% plasma cells. A presumptive diagnosis of light chain myeloma was made. Histopathologic exami-

Correspondence to: Lutfiye Bilge Caliskan, Department of Internal Medicine, Tepecik Educational and Research Hospital, Izmir, Turkey; E-mail: harunakar.md@gmail.com

nation of the bone marrow biopsy revealed multiple myeloma that was light chain myeloma and the patient was referred to a hematologist and the light chain myeloma scheduled for chemotherapy. The renal function improved and the patient was free of dialysis 6 months after the diagnosis.

Discussion

This case shows the importance to fully explore the underlying cause of acute renal failure. Morerover, treatment should be directed against the primary disease [5,6]. The patient described in this case presented with renal failure of unknown etiology. Acute renal failure may be one of the types of presentation of multiple myeloma. In internal medicine or nephrology clinics, MM may present with renal dysfunction of unknown etiology or acute renal failure. If the underlying cause of acute renal failure is thought to be multiple myeloma, it is easily diagnosed by serum M protein, hypercalcemia, anemia, increased erythrocyte sedimentation rate, albumin-globulin reversal, skeletal pain or typical bone lesions. In some patients the diagnosis will be evident from careful examination of a peripheral blood smear.

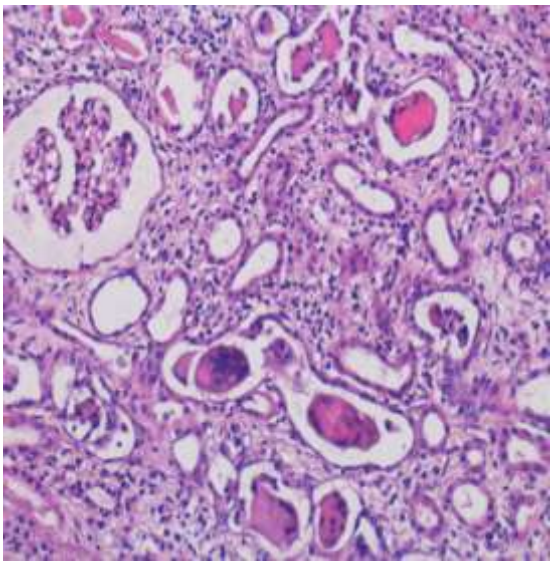

Fig. 1. Renal tubules filled with eosinophilic fractured casts in renal biopsy (H&Ex200)

On the other hand, the absence of lytic lesion, back pain and hypercalcemia makes the diagnosis of a plasma cell dyscrasia difficult. This patient had no lytic lesions, back pain or hypercalcemia. Rarely, some cases with multiple myeloma can be diagnosed by the findings of a renal biopsy without typical clinical presentation [7,8]. Border *et al*. reported that renal biopsy findings in four cases revealed typical diagnostic features of "myeloma kidney" and confirmed the diagnosis by bone marrow examination [8]. As a possible mechanism, it is suggested that tubular obstruction and retrograde urine flow precedes the development of "myeloma kidney" and acute renal failure [8]. The patient's renal biopsy, immunofixation electrophoreses, bone marrow aspiration and biopsy seem most consistent with MM. This patient developed intrinsic acute renal failure secondary to intrarenal tubular obstructions with myeloma proteins and required temporary support by hemodialysis. In the patient's renal biopsy the renal tubules were filled with eosinophilic fractured casts (Figure 1) whereas there were multinucleated histocytic giant cells around renal tubules (Figure 2). Circulating free light chains (FLC) could lead to acute renal injury due to intratubular precipitation, and initial treatment can be achieved by adequate hydration and removal of the FLC with different apheresis techniques [9]. In addition to specific myeloma chemotherapy, prolonged hemodialysis sessions had been reported to be an effective treatment for cast nephropathy [9]. Early onset of treatment could be a decisive factor for the response. On the other hand, impaired renal function has been described as a marker of poor prognosis and patients' survival is predicted more by the reversibility of renal damage associated with MM [10].

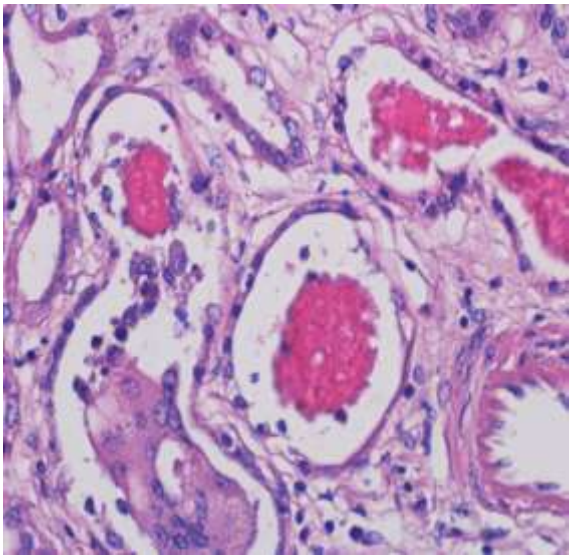

Fig. 2. There are multinucleated histocytic giant cells around renal tubules in renal biopsy (H&Ex400)

This case illustrates several important points. Acute renal failure due to multiple myeloma is rare, but it may rarely be a characteristic of the disease. It is crucial that all patients with renal failure of unknown etiology should be screened for possible plasma cell dyscrasia. The workup of a patient with renal failure of unknown etiology should include immunofixation electrophoreses. Other diagnostic tests may be necessary to confirm the diagnosis such as biopsies. The possibility of an underlying multiple myeloma in the case of acute renal failure and anemia of unknown origin should be considered.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 92-94

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Case report

A Case of Hypercalcemia after Thyroidectomy

Irini Katsarou[1], Ourania Drakoulongona[2], Antonis Christopoulos[3] and Goula Konstantina[2]

[1]Renal Unit, General Hospital Zakynthos, [2]Renal Unit, St Andrews' General Hospital Patras, [3]Doctor's Hospital Athens, Greece

Abstract

Total thyroidectomy is complicated by hypoparathyroidism in 1-3% of patients. Hypoparathyroidism is treated with oral calcium and vitamin-D supplements. Everyday use of calcium and vitamin D can lead sometimes to hypercalcemia. Ingestion of large amounts of calcium and absorbable alkali that cause hypercalcemia, various degrees of renal failure, and metabolic alkalosis, can be associated with a diagnosis of calcium-alkali syndrome. This syndrome was first identified as milk-alkali syndrome, after treatment of peptic ulcer disease with milk and alkali which was widely adopted at the beginning of the 20th century. With the introduction of histamine-2 blockers and proton pump inhibitors, the occurrence of milk-alkali syndrome became rare; however, it has emerged recently as calcium-alkali syndrome because of the wide availability and increasing use of calcium carbonate, mostly for osteoporosis prevention. We present a female patient with hypoparathyroidism who presented with hypercalcemia and alkalosis as a result of treatment with calcium carbonate, vitamin D and thiazide diuretic. The patient was treated successfully by discontinuation of the above drugs, intravenous fluid administration and enhancement of calcium renal excretion. Hypercalcemia presenting as calcium-alkali syndrome is a diagnosis that requires a high index of suspicion in order to quickly identify the disorder and initiate appropriate therapy. It is important for clinicians to keep the syndrome on their list of differential diagnosis.

Keywords: hypercalcemia, hypoparathyroidism, calcium-alkali syndrome, vitamin D

Introduction

Total thyroidectomy is complicated by permanent hypoparathyroidism in a small percentage of patients. It's therapy consists of long-life treatment with calcium supplements (1.5-3 g/day) and active metabolite of vitamin D (0.5-2 μg/day).

Daily use of calcium with vitamin D can sometimes lead to hypercalcemia. Hypercalcemia is defined as an increase in total serum calcium >10.5 mg/dl (>2.5 mmol/lt) or ionized calcium >5.6 mg/dl (>1.4 mmol/lt). An increase in total calcium over 14 mg/dl (3.5 mmol/lt) is characterized as serious hypercalcemia-hypercalcemic crisis.

Calcium-alkali syndrome consists of hypercalcemia, alkalosis and renal failure. Recently many patients present with this diagnosis and it is the third commonest cause of hypercalcemia [1,2].

We report a case of a female patient with total thyroidectomy and permanent hypoparathyroidism who presented with symptomatic hypercalcemia.

Case report

A 59-year-old female patient presented to the Emergency Department complaining on vomiting over the last 5 days, muscle ache and weakness. No fever or other symptoms were reported. From her past history the patient reported papillary thyroid cancer that was treated by thyroidectomy 20 years ago. She developed postoperative hypoparathyroidism and remained on life-long treatment with thyroxin 125 mg/day and calcium lactate gluconate and calcium carbonate. Although on calcium supplementation, the patient was frequently admitted to the Department of Internal Medicine of our Hospital because of tetanic crises. During her previous admission (one month ago), alphacalcidol (3g/24h) and hydrochlorothiazide 25 mg/24h were added to her drugs whereas the dose of calcium carbonate was also increased from 1 g to 3 g/24h.

On physical examination, arterial blood pressure was 110/70 mmHg and heart rate 75/min. No swelling or signs of dehydration were noted whereas the clinical examination of lungs, heart and abdomen showed no pathologic findings. No palpable lymph nodes were found. The ECG was normal.

Laboratory tests showed severe hypercalcemia [Ca: 18.4 mg/dl (normal range 8.5-10.1 mg/dl)], impaired renal function (serum creatinine: 2.8 mg/dl), hypokalemia (K: 2.8 mmol/lt), normocytic anemia (Hb: 10.4 g/dl, Hct: 31.2%, MCV: 89), elevated CRP levels (7.3 mg/dl) and hyperuricemia (uric acid: 14.4 mg/dl). Sodium, chloride and proteins in blood were in normal range (Na: 135 mmol/lt, Cl: 100 mmol/lt, albumin: 4.2g/dl).

Correspondence to: Konstantina Goula, Renal Unit, St Andrews' General Hospital Patras, Greece; Phone: 00 30 261 022 7960; E-mail: kgoula1@hotmail.com

An ultrasound scan showed normal size and echogenicity of kidneys. A CT of the abdomen and lungs was also without any abnormality. PTH blood levels were low [PTH: 3 pg/ml (normal range 10-93)] whereas thyroid hormone and alkaline phosphatase blood levels were within normal range.

After admission, the patient had polyuria (6800 ml/day) with a urine specific gravity of 1003-1004 and urine pH 7-7.5 (Table 1).

Table 1. Laboratory tests

Day	Na (mmol/L)	K (mmol/L)	Urea (mg/dL)	Cr (mg/dL)	Ca (mg/dL)	Uric acid (mg/dL)	Urine output ml
1	126	2.8	115.8	2.8	17.7	14.4	5400
2	135	3.4	108	2.9	18.4	10.4	6800
3	140	3,4	93.9	2.3	12.4	8.7	4200
5	141	3.0	82	2.2	10.3	7.1	3400
7	142	3.8	44.7	1.7	9.2	7.0	2500
8	143	3.8	45	1.7	7.8	6.8	2200

Hypercalcemia was managed with cessation of calcium and vitamin D supplements, administration of sodium chloride solutions and furosemide (20 mg/12hr intravenously). Hypokalemia was treated with potassium supplements. The patient was discharged 8 days after her admission in a good clinical condition with calcium levels of 7.8 mg/dl and improved renal function (serum creatinine: 1.7 mg/dl and GFR: 49.8 ml/min).

Discussion

Hypercalcemia is characterized by high calcium concentration in the blood which sometimes can lead to fatal complications. The manifestations of mild hypercalcemia are weakness and fatigue but as calcium levels increase further, symptoms become more serious. The level of calcium in the blood along with the rate of development of hypercalcemia are related with symptoms from central nervous system, gastrointestinal tract, heart and kidneys [3]. Stupor, weakness, confusion and coma represent serious symptoms from central nervous system whereas constipation, nausea, vomiting and lack of appetite are symptoms of hypercalcemia from the gastrointestinal tract. Moreover, patients with hypercalcemia can develop peptic ulcers and pancreatitis. Cardiac arrhythmias, hypotension and short QT interval in the ECG represent heart involvement whereas polyuria, dehydration and thirst along with presence of kidney stones and renal failure represent involvement of urinary tract.

In our patient, hypercalcemia was probably related to the administration of calcium sparing drugs alphacalcidol (3 mg/day), calcium carbonate (3000 mg) and diuretics (hydrochlorothiazide) in high doses. Calcium carbonate, which contains alkaline ions, and hydrochlorothiazide probably contributed to metabolic alkalosis whereas vomiting further exacerbated hypercalcemia and metabolic alkalosis by loss of hydrogen cations from gastrointestinal tract and contraction of intravascular volume.

Diuretics represent the most common cause of drug-induced metabolic alkalosis. This is probably related to increased excretion of chloride in the urine that leads to intravascular volume depletion. The concentration of bicarbonate increases because of reduced volume of distribution (contraction alkalosis) leading to mild metabolic alkalosis. Hypercalcemia due to thiazide diuretics is uncommon in patients without other comorbidities. It causes vasoconstriction of the afferent arteriole leading to decline of GFR as well as activation of calcium sensing receptor (CaSR) in the thick ascending limb of the loop of Henle leading to deactivation of Na-K-2Cl co-transporter that is deactivated with final result natriuresis and hypovolemia. Hypovolemia leads to increased bicarbonate reabsorption and alkalosis that further aggravates hypercalcemia (by activating a calcium channel in the distal convoluted tubule and in the colon resulting in increased calcium reabsorption) [4,5].

As our patient ingested a high dose of calcium and absorbable alkali (calcium carbonate), we thought that the patient could have calcium-alkali syndrome. This syndrome presented in the past as milk-alkali syndrome. Milk-alkali syndrome is characterized by hypercalcemia, renal failure and metabolic alkalosis [6]. Leo Hardt and Andrew Rivers in 1923 first reported the complications of Sippy diet treatment (Bertram W. Sippy 1866-1924) which consisted of great quantities of milk and calcium for peptic ulcer treatment. They correlated Sippy diet with renal failure and metabolic alkalosis [7,8]. Thirteen years later, Cuthbert Cope recognized also hypercalcemia as part of the syndrome, thus completing the classic triad [2]. After the introduction of H2-antagonists and proton pump inhibitors for the treatment of peptic ulcer, the incidence of the syndrome declined and it became a rare cause of hypercalcemia [9,10]. Currently it has reemerged as calcium-alkali syndrome that represents the third commonest cause of hypercalcemia, due to the wide use of calcium carbonate and vitamin D for prevention and treatment of osteoporosis [2,11].

In our case, we treated the patient's hypercalcemia by discontinuation of the responsible drugs fluid administration and furosemide in order to enhance renal excretion of calcium. Intravenous fluid and furosemide were enough for restoring calcium levels; however, renal function did not recover completely (creatinine clearance: 49.8 ml/min). Similarly to our patient, cases with

permanent functional and structural kidney damage have been described in the literature [12,13].

Conclusion

In conclusion, patients with predisposing factors for development of hypercalcemia such as renal failure and thiazide diuretic use should be closely monitored with frequent measurements of calcium blood levels when treated with calcium carbonate and vitamin D. Physicians should also consider the diagnosis of calcium-alkali syndrome for such patients, since it is a common cause of hypercalcemia.

Conflict of interest statement. None declared.

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BANTAO Journal 2016; 14(2): 95-97

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Case report

Psoas as an Unusual and Overlooked Place for a Metastatic Tumor

Bengu Erkul[1], Bahar Engin[1], Mehmet Can Ugur[1], Sumeyye Ekmekci[2], Emrah Akay[3] and Harun Akar[1]

[1]Department of Internal Medicine, [2]Department of Pathology, [3]Department of Radiology, Tepecik Education and Research Hospital, Izmir, Turkey

Abstract

We report a case of a 60-year-old hemodialysis patient who clinically mimicked psoas abscess, which was subsequently proven to be from metastatic disease secondary to uroepithelial tumor. The patient presented with 3 weeks history of fever, weight loss and back pain. Computer tomography (CT) scan of abdomen and pelvis revealed psoas muscle infiltration not amenable to drainage by interventional radiology. Careful history to provide additional clues to the diagnosis is of paramount importance in this condition.

Keywords: psoas, metastatic tumor, dialysis

Introduction

Psoas is generally associated with infectious process as abscess formation. Other pathologies such as malignancy are rare. In these conditions, diagnosis is often delayed by misinterpretation as infectious diseases, urological or abdominal disorders. Despite recent advances in medicine, differential diagnosis of psoas pathologies is still a diagnostic problem. Our patient is a 60-year-old man with a history of low grade uroepithelial papillary carcinoma who presented with a chief complaint of fever initially noted twenty days prior to his admission to the hospital.

Case

A 60-year-old hemodialysis patient came to the emergency room with complaints of fever, weight loss and back pain. Further history revealed that he had a history of nephrectomy five years ago due to a low-grade uroepithelial papillary carcinoma. Since he was unable to continue functioning independently, he was admitted to the Internal Medicine Clinic for further evaluation. He was alert and oriented person with a temperature of 38.3ºC, a pulse of 74 bpm and blood pressure of 150/82 mm Hg. The abdomen was soft and non-tender. Head, ears, eyes, nose and throat examination was unremarkable. Laboratory analyses revealed the following: WBC count 12400/µl with 28.2%, 8.9% lymphocytes. The hemoglobin and hematocrit were 10.6 g/dl and 32.6%, respectively, with a platelet count of 442000 /µl. The blood chemistry profile revealed an aspartate aminotransferase (AST) of 11 units/L and alanine aminotransferase (ALT) of 5 units/L, LDH of 333 units/L, alkaline phosphatase of 98 IU/L and total bilirubin of 0.5 mg/dl. Erythrocyte sedimentation rate was elevated at 84 mm/h, C-reactive protein (CRP) 23.8 mg/dl, adenosine deaminase (ADA) 42. Other tests of autoimmunity such as antinuclear antibody (ANA) and complement levels were in normal range. The initial blood cultures did not grow any pathogens. Purified protein derivative (PPD) was anergic. A transthoracic echocardiogram revealed no vegetations. The patient completed 14 days of broad spectrum antibiotic therapy and did not improve clinically. The patient's CT scan was consistent with a malignant process (Figure 1). A huge soft tissue mass which was about 108*73 mm was seen in the right paravertebral part of the abdomen. The mass could not be distinguished whether it was solid or in abscess formation. Multiple lymph

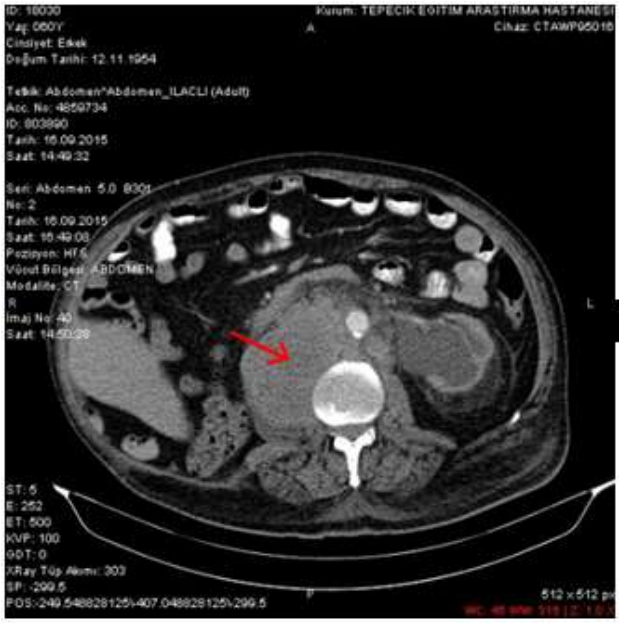

Fig. 1. Red arrow shows a huge soft tissue mass which was about 108*73 mm seen in the right paravertebral part of the abdomen

Correspondence to: Mehmet Can Ugur, Izmir Tepecik Education and Training Hospital, Department of Internal Medicine, Izmir, Turkey; E-mail: harunakar.md@gmail.com

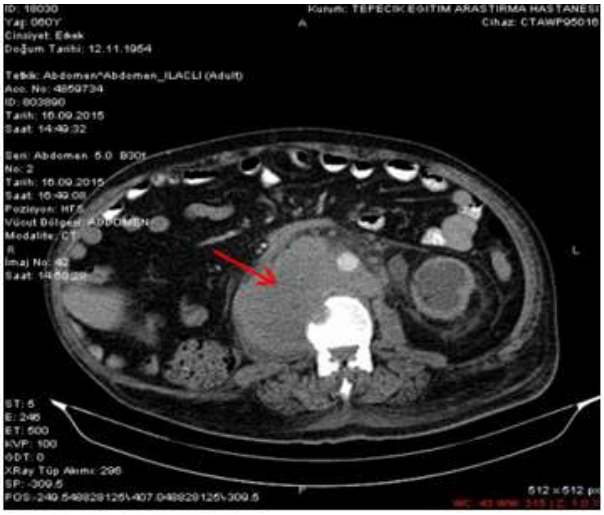

Fig 2. Red arrow shows multiple destructive lumbar vertebral lesions with associated soft tissue mass involving right psoas muscle

nodes were reported in paraaortic and paracaval area (Figure 2). The patient then underwent a positron emission tomography (PET/CT), which revealed multiple destructive lumbar vertebral lesions with associated soft tissue mass involving right psoas muscle. PET/CT showed psoas muscle infiltration or vertebral metastases. Diagnosis was made by a fine needle aspiration biopsy from the soft tissue mass in the right paravertebral area. The pathology was reported as metastatic uroepithelial adenocarcinoma. The patient was transferred to the Oncology Clinic for treatment of metastatic uroepithelial adenocarcinoma.

Discussion

The psoas is a retroperitoneal muscle that lies in close proximity to anatomic structures as sigmoid colon, jejunum, appendix, ureters, aorta, renal pelvis, pancreas, iliac lymph nodes, and spine [1]. Thus, infections in these organs can contiguously spread to the psoas muscle. The psoas muscle has a rich vascular supply that is believed to predispose it to hematogenous spread from sites of occult disease. A primary psoas abscess (PA) occurs from hematogenous dissemination of a distant infection [1,2,3]. A secondary abscess arises by contiguous spread of a local infective process and inflammatory or neoplastic diseases of the bowel, kidney and spine, such as Crohn's disease and appendicitis [3].

Psoas abscess is a rare situation with a nonstable and non-unique clinical signs which makes it easier to misdiagnose or to make late diagnosis. The presentation of a psoas abscess is commonly seen in conjunction with infection, especially tuberculosis. Metastasis to the psoas muscle and tumoral involvement of the psoas muscles is rare [4]. Avery reported a patient with a psoas lesion 4 years after a sigmoid colectomy for adenocarcinoma. Radiological appearances were thought to be typical of an abscess, but an attempted aspiration was unsuccessful. A biopsy was taken and histology showed metastatic adenocarcinoma [5]. Yap *et al*. reported a case of metastasis from transitional cell carcinoma of the bladder to the body of the psoas muscle masquerading as psoas abscess [6]. Repeated CT scan showed ill-defined low-density area with inflammatory changes involving the right psoas muscle. In this report, a fine needle aspiration biopsy of the right psoas was performed by CT guidance to obtain the histopathologic diagnosis [7]. Singh *et al*. reported a 68-year-old male patient presented with fever, groin pain, leukocytosis and azotemia mimicking pyelonephritis that was subsequently proven to be from a diffuse, large, B-cell lymphoma by fine-needle aspiration cytology and biopsy from the lesions. They reported that computed tomography revealed a bulky right psoas muscle, enlarged right kidney with thickening and enhancement of walls of pelvicalyceal system and perinephric fat stranding [8].

The patient presented here is a 60-year-old man who was in his usual state of health until 3 weeks prior to admission when he developed fever, poor appetite, weight loss and back pain. The patient's symptoms of fever, weight loss, back pain and difficulty in walking persisted despite two-week course of antibiotics. The spectrum of the differential diagnosis is broad and should include infectious and noninfectious causes. While the initial clinical suspicion was intra-abdominal abscess, failure of the symptoms to resolve after convenient antibiotic therapy suggested that another disease or pathogen might be responsible for the situation. The elevated ESR suggested an occult inflammatory condition or underlying neoplastic process. The most important entities to consider are psoas abscess and tumoral invasion. Mycobacterium tuberculosis should also be considered, negative ARB argue against a mycobacterial etiology. The patient had a history of nephrectomy five years ago due to low-grade uroepithelial papillary carcinoma. This is an important clue to the diagnosis. The patient history and presentation seem most consistent with tumoral invasion. Psoas muscle metastasis in this elderly hemodialysis patient is likely to be caused by uroepithelial tumor. In most cases the diagnosis may be secured by interventional radiology currently.

Knockaert *et al.* evaluated 47 patients, older than 65 years, meeting the criteria of FUO [9]. Infections, tumors and multisystem diseases were reported in 25%, 12% and 31% of the patients, respectively. The percentage of malignant diseases was found to be higher in their elderly patients than in the younger ones. Knockaert *et al.* suggested that multisystem diseases such as temporal arteritis occurred as the most frequent cause of FUO in the elderly, and infections, particularly tuberculosis, remain an important group.

Conclusion

In this case, a patient with a diagnosis difficulty should start with a careful history to provide additional clues to the diagnosis. It merits to be emphasized malignant etiology should be ruled out in a similar situation.

Conflict of interest statement. None declared.

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