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*Editorial***Organ Donation in India from the Point of Liver Specialists**Vaishaly Bharambe¹, Purushottam R Manvikar², Sheetal Mahajani³ and Bipin Vibhute⁴

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Abstract

Introduction. Organ transplantation surgeries are best mode of treatment for patients in organ failure. However, acute shortage of organs has deprived thousands of patients of benefits of this surgery. Mortality in case of patients on waiting list for liver transplant is more than in cases of kidney transplant because while kidney failure patient can be supported by dialysis, liver failure patient has no such alternative.

Methods. The study consisted of detailed discussion with liver specialists regarding their experiences related to organ donation in cities in Maharashtra state of India. The discussions were analyzed for insights into the organ donation.

Results. 12 liver specialists participated in the study. The analysis of the discussion revealed themes such as awareness of categories of donors, brain death, and lack of diagnosis of brain death.

Conclusion. The liver specialists suggested that people of this region lacked awareness about categories of organ donors and about concept of brain death. There was lack of diagnosis of brain death by doctors of various hospitals. They suggested that a common brain death committee be created per region to be called upon for brain death diagnose at those hospitals which did not have a brain death committee. There was need for more hospitals to be sanctioned for transplant surgeries and to sensitize the police personnel towards organ donation. They all stated that some form of non-monetary incentive had to be worked into the system to benefit the donor or the family of the donor.

Keywords: organ donation, liver transplant, hepatologists

Introduction

Organ transplantation was one of the greatest technological achievements of modern medicine. The benefits of this achievement are limited by the shortage of trans-

plantable organs [1]. This organ shortage has deprived thousands of patients of a better quality of life as well as increased the expenditure of patients on alternative medical care [2]. World over, there is increasing awareness regarding organ failure and transplantation as a possible treatment option for it. The demand for organs for transplantation is hence rapidly increasing all over the world [3].

In India every year 10⁵ people die awaiting an organ for transplant. There is a wide gap between demand for organs and the actual availability of the organs for purpose of transplant [4]. In 2017 when the demand for kidneys in India was estimated to be 200000, about 10000 kidneys became available by process of organ donation. The similar demand for liver transplant in the same year resulted in actual available livers being found to be only 700 [5]. Thus, a huge gap exists between demand and supply of organs for transplant in India [6]. Authors have reported that, mortality in case of patients on waiting list for liver transplant is more than in cases of kidney transplant because while kidney failure patient can be supported by dialysis, liver failure patient has no such alternative. Also, every deceased donor provides two kidneys to be available in the donor pool, while the number of livers contributed by one donor is still single [7].

Hence the present study was undertaken to obtain the perspective regarding organ donation from point of view of liver specialists in Maharashtra, India.

The presented study aimed to assess the experiences of liver specialists from point of view of organ donation. Afterwards the findings were reviewed and possible solutions were suggested to improve the rate of organ donation in India.

Material and methods

The following study was carried out in the cities of Maharashtra, (a State in India) which had registered organ transplant centers or had a non-transplant organ retrieval center (NTORC). Institutes Ethical committee

clearance was obtained before starting the study. It consisted of discussions with liver specialists regarding their experiences in relation to organ donation. The study also attempted to bring out solutions as suggested by the participants in relation to these challenges. The inclusion criterion for the study population was that all participants had to be liver specialists residing in those cities of Maharashtra which had facility to carry out organ transplant surgeries or which had a non-transplant organ retrieval center. Only those liver specialists consenting to participate were involved in the study. The exclusion criterion was those who declined to participate in the study. The sampling method used here was convenience sampling. The liver specialists were requested for time to discuss challenges involved in process of organ donation and to share their experiences in the field of organ donation. The respondents were assured

that confidentiality of identity would be maintained and ethical principles would be followed. The interviews were noted down during the discussion and the suggestions of the liver specialists were also noted. The discussions were analyzed thereafter, for the themes generated during the discussions and for insights into the organ donation activity. This qualitative research was done till the point of saturation.

Results

A total of 12 hepatic specialists were invited for participation in the study. 5 were hepatologists while 7 were medical professionals trained in liver transplant surgeries. All accepted to participate in the study. The themes generated during the discussion are seen in Figure 1.

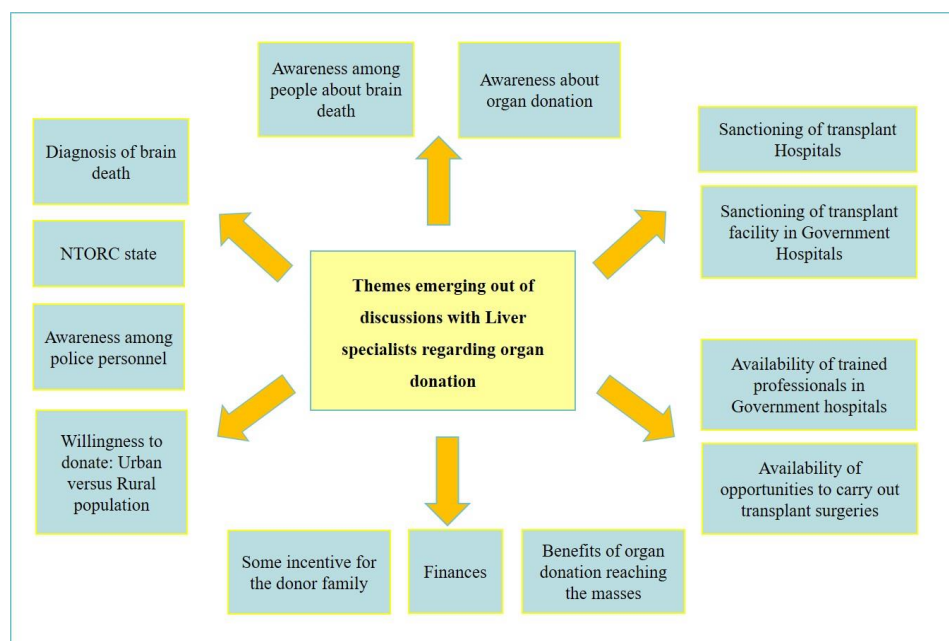


Fig 1. Themes generated during discussion with Liver specialists

Discussion

Organ donation is a process by which a person allows removal of a functioning organ from their body, its surgical transport into another person's body and the organ then serves the function of the new body [8]. The person giving the organ is referred to as the donor and the person receiving the organ is referred to as the recipient [8]. This can occur either with the donor being alive with his or her consent or it can occur with the person being brain dead or cardiac dead with the family member's consent. The recipient is always in organ failure of the organ which is being transplanted [9]. Common organs or tissues being transplanted today are kidneys, liver, heart, lungs, cornea etc. [10]. While few of these organs such as one kidney or a part of

liver can be donated while the donor is alive, most of the organs are donated after the donor's death [8].

In the world today millions of people are in organ failure, awaiting an organ for transplant. In USA in 2019, 120000 people were awaiting an organ for transplant [11,12].

Due to significant advances in the techniques of dialysis the patients with end stage renal disease can survive longer than before. Thus, patients awaiting kidneys for transplant keeps increasing.

However, in case of liver failure no such option of dialysis is available today. Hence, mortality in case of patients on waiting list for liver transplant is even more than that in cases of kidney transplant as liver failure has no such alternative of dialysis. Also, while every deceased donor provides two kidneys available

in the donor pool, the number of livers contributed by one donor is still single [13].

The first successful liver transplant was carried out in 1968. Since then liver transplant has become a standard therapeutic option for patients with chronic liver disease and for patients with acute liver failure. Newer surgical techniques and better immunosuppressants have further improved the success rate of this surgery. However, the availability of organs is unable to meet the demand for the organs for transplant [12].

In 1988, split liver transplant was carried out. This allows one liver to be split into two recipients thus providing transplant support to two needy patients [14]. However, this is a technologically challenging procedure.

India has huge number of patients who have liver failure. Today the demand for liver transplant is about 20 per million population. In 2017 the entire country yielded 905 deceased donations resulting in total of 2500 organ transplant surgeries [15]. Thus, the demand is far more than the supply of livers and many patients (almost 50%) die on the waiting list for suitable donor for transplant.

The journey of the ill patient in imminent liver failure usually begins by approaching the hepatologist for health issues. The hepatologists help maintain the health of the patient, often keeping him or her alive while they are on the waiting list for a cadaver organ or till the transplant of liver from a live organ donor is carried out by the liver transplant surgeon. During this period the liver specialists face several challenges in relation to organ donation. Hence the present study was undertaken to get an insight into these challenges from the liver specialist's perspective. The discussion with the liver specialists is being presented as per the themes generated during the discussions. Some of the themes are grouped together for purpose of ease of discussion.

Theme: Awareness among people about organ donation; Awareness among people about brain death

The participants related that the people of this region were aware of organ donation as a concept. However, they lacked understanding of relevant details in this matter which often resulted in poor organ donation rate in this region. A similar finding was reported by Bharambe *et al* in 2018. The authors stated that people of this region lacked understanding about categories of organ donors and the organs that could be donated by each category of donor [16].

In this regard, during the discussion the liver specialists stated that the Maharashtra state of India saw establishment of Zonal transplant coordination center (ZTCC) in the year 2000. This was a nongovernment organization (NGO) set up to promote and facilitate organ donation in every way possible [17]. Initially there was only one ZTCC. Today there are 4 ZTCCs in Maharashtra state i.e. ZTCC of Mumbai, Pune, Nagpur and Auranga-

bad regions respectively. The ZTCCs began a number of steps which would improve organ donation rates and associated transplants in the area under their jurisdiction.

The ZTCCs planned a number of strategies to promote organ donation. Their primary target was to increase awareness among the masses regarding organ donation and brain death by way of consistent campaigning and public education exercises. They planned to effectively use the media for this purpose [18]. While the media in earlier days gave publicity to the negative side of organ donation and transplants, revealing the various scams related to transplants, the effects of this publicity hurt the transplant programme in India by preventing authentic donors from coming forward fearing misuse of their donated organs. Now the same media was used to promote organ donation, publicizing every successful organ donation and donor [19,20]. The negative publicity was discouraged and positive true stories were regularly published to change the image of organ donation.

Recipients and their family members were encouraged to become spokespersons for the cause of organ donation so that people would be assured that what they gave in the form of organ donation, was benefitting their own people [21].

Films were created and shown to people to further educate the masses about organ donation activity [22].

The liver specialists felt that besides use of media, all hospitals, cinema theatres, malls and other public places should also be used to promote organ donation.

Theme: Diagnosis of brain death

The liver specialists observed that the biggest challenge to organ donation related to deceased donor programme in India is "diagnosis of brain death". The hospitals in Maharashtra, India were not prioritizing identification and maintenance of brain-dead donors.

The Transplant of Human Organs Act (THO Act), India, defines "brain-stem death" as the stage at which all functions of the brain-stem have permanently and irreversibly ceased and has been so certified [23].

Observing that the brain death was not being declared promptly, Public Health Department of Government of Maharashtra issued orders making it mandatory to declare "brain-death" and certify it, and the certification of the same be conveyed to the Zonal Transplantation coordination committee for distribution of the organs [24].

The liver specialists observed that despite the above mandate by which declaration of brain death was made mandatory, brain death was not being diagnosed. They stated that, this was probably because this is a "directive" and is perceived as not punishable. It is not a "law". If it were made into a law, not following which may result in action against the hospital, brain death diagnosis will definitely increase [25].

The participants made several other suggestions to overcome the problem of non-diagnosis of brain death.

They suggested that the hospitals could have an internal audit. If the patient is dead, the case could be discussed. The chances of that patient having been brain dead could be probed. This could be followed up with a probe into reasons for non-declaration of brain death or for not seeking a neurological opinion in this regard. A study probing into neurological cases admitted in the ICU in Bangladesh have found that there is no dearth of brain dead cases in ICUs but there is a need to promote and popularize the organ donation from the brain dead individual and also to familiarize the faculty with procedure of brain death declaration [26].

The participants discussed that there may be situations wherein the patient was admitted at a hospital that is neither a non-transplant organ retrieval center (NTORC) nor an established center for transplant surgery. In this case, there would be no authorized personnel to declare brain death. Nagral and Amalorpavanathan have discussed this situation and how it acts as a deterrent to diagnosis of brain death. They went on to describe how in such situation cadaver donors had to be shifted to recognized transplant hospitals solely with purpose of organ retrieval. This led to establishment of NTORCs [27].

The participants in the present study suggested that in such cases when the hospital did not have their own established and recognized brain death committee to declare brain death, the committee from a nearby authorized hospital could be asked to examine the patient for diagnosis of brain death. The patient may alternatively be also shifted to an authorized hospital. The interim charge of moving the patient may be borne by the Hospital that finally receives the donated organ. Nagral and Amalorpavanathan discussed this situation as being advantageous to the hospital where the cadaver donor would be transferred as that hospital would now have advantage of allocation of organs on priority as an "in house" donor [27].

The participants suggested that problem of diagnosis of brain death in the absence of brain death committee, could be solved by having a State government recognized brain death committee in every city where there are hospitals sanctioned for organ transplant. This committee could be kept "on-call" for diagnosis of brain death in that region. Just like ZTCC serves a region, coordinating the organ donation activities of that region, similarly this team would respond to the requirement for examining any patient suspected of being brain dead in their respective region or city. This would prevent the necessity to move the patient to another hospital which has a brain death committee approved by ZTCC.

In 1968, United States of America (USA) saw the establishment of organ procurement organizations (OPOs) in different regions and states. They were accorded the job of procuring deceased donor organs for organ transplants in their particular regions. They were to remain in contact with hospitals that were conducting retrieval and transplant surgeries as well as with the family of the

deceased person. They were to attend audits regarding patients in USA who could be brain dead but were not tested for brain death or whose family was not approached for organ donation etc. Thus, every death got discussed and missed declaration of brain death got probed [28].

Theme: NTORC

The liver specialists discussed problems regarding Hospitals getting status of non-transplant organ retrieval center (NTORC). They said that, many hospitals were reluctant to apply for this status. The hospital authorities believed that such a status would mean that they would be involved in retrieval of organs with the tag of patient "death" attached to them while the actual transplant surgeries took place at another hospital. Thus, though they were involved in a very positive activity, the resultant publicity and history of subsequent death of the patient may harm the reputation of the hospital by public misconception.

Theme: Awareness levels among the police personnel

The hepatologists reported that to carry out cadaver organ donations, there was a need for no-objection certificate from the police. Untrained police personnel often delayed the nod, leading to delay of organ donation or sometimes loss of the patient and the opportunity for organ donation. They therefore believed that there was a necessity to sensitize them about organ donation and its various aspects in relation to the sphere of activity of the police personnel.

Recently the **Maharashtra State's organ transplant cell** is considering a proposal for a separate organ transplant module for the police during their training sessions, to sensitize them. Discussions will be held with Police personnel educating them about new definition of death i.e. death with a beating heart and the cooperation needed from the police for success of the organ donation activity. They will also be explained the importance of arrangement of the green corridor [29].

Theme: Sanctioning of transplant hospitals

The participants stated that previously, affording patients who were in organ failure, were often going to southern states of India and registering there for liver transplants. It took a long time for the government to sanction the liver transplant facility in hospitals in this region. As stated earlier, a patient with a severe liver ailment does not have much time to wait for the transplant surgery [30]. Hence the participants suggested that more hospitals should be given sanction for carrying out transplant surgeries. A hospital that invests into the set up needed for these surgeries and employs necessary staff on their payroll is under pressure to now carry out the transplant surgeries. They would

therefore promote organ donation in that region. The entire area would benefit by the resultant awareness programs run by the hospital, the brain deaths declared, the organs subsequently retrieved and the transplant surgeries that will be conducted.

Theme: Sanctioning of transplant facility in Government hospitals

The organ donation movement has been perceived as "taking from poor and giving to the affording or rich". It has been observed that the wealthy can leverage their socioeconomic status to gain access to organs [31]. To counter the effect of economic status on the organ donation movement, the participants felt that more government hospitals should apply for a sanction for carrying out transplant surgeries. The government hospitals receive a number of cases which could be brain dead. With these hospitals becoming a part of the transplant surgery group in the region, the number of brain death declarations may increase leading to rise in number of available organs. Thus the poor patients on the waiting list could get benefit of transplant surgeries without the pressure of financial constraints. In 2018, Sassoon Hospital in Pune became the first government hospital to carry out liver transplant on a patient who had been suffering from liver failure but unable to undergo transplant surgery due to the expenses involved. Thus, the facility of liver transplant becoming available at this Hospital directly benefitted the patient of lower economic status [32].

The liver specialists revealed that the ZTCC may be amending their guidelines of organ allocation so that organs from the government hospitals could be given preferentially to government hospitals and be offered to others only thereafter. The final result of this would be easier availability of benefits of transplant surgeries to the poor. In 2006 the Indian Army started the Armed forces organ retrieval and transplantation authority (AORTA). This was responsible for sensitizing the armed forces towards brain death and organ donation. 53 patients underwent liver transplant in the military hospital in New Delhi from 2007 till 2011. This included live and deceased donor transplants. The survival rates as well as the morbidity rates were comparable with the international data [33].

State government and Military hospitals can thus be a solution to the problem of prohibitive expense of liver transplants, thus helping to reach benefits of this procedure to the masses.

Theme: Availability of trained doctors in Government Hospitals

The government hospitals often do not have qualified liver transplant surgeons. The participants described how a medical professional completes training in their

specialty in India or abroad and is expected to join the Government at the lowest level of assistant professor often working under persons less qualified than themselves. In these conditions at the kind of salary offered, it was financially not feasible to work in the government sector in this field. They suggested that the government could send their own employees for specialization in liver related ailments following which they would then develop and successfully run the liver transplant programme in the government hospitals.

Theme: Availability of opportunities to carry out transplant surgeries

The liver transplant surgeons opined that liver transplant surgery is a dependant branch. It depends on the availability of a suitable live donor or on declaration of brain death and existence of the necessary infrastructure for this surgery. Many surgeons who have undergone rigorous training (in India or abroad), have had to subsist by doing general surgery for years after returning to India, awaiting opportunity to carry out the transplant surgeries for which they have trained. This can be very demoralizing, causing many to consider not returning to India and making a career in some other country where there is enough work in their field of choice. This is an example of "paucity in the midst of plenty", i.e. there is so much transplant related work needed in India, but not enough means of carrying out this work. In a study conducted in USA, it was observed that out of the total surgeons undergoing fellowship training in transplant surgery, 12% did not find transplant jobs and 14% did not get to transplant the organ of their choice [34]. A rise in the rate of organ donation would increase the number of organs available for transplant and thus would increase the opportunities for these surgeons to carry out transplant surgeries they were trained to conduct.

Theme: Finances

The participants observed that it is the poorer class that agrees to organ donation more easily than the rich and educated. A research in USA has found that when there are equally sick patients, it is more likely that the rich patient will get a transplant compared to the poor. This could be because rich patient can afford to be on multiple waiting lists of different states but this option was not available to the poor patient. Also, not all insurance policies cover the additional testing needed to get a second listing in multiple hospitals and states [35]. Money thus begins to influence organ donation activity in various ways. It can become the driving force behind the organ donation activity in a positive way too. As mentioned earlier, a hospital which is sanctioned for liver transplant has invested a lot in setting up the infrastructure needed for these transplant surgeries and every liver transplant surgery performed would help

recovery of this investment. As a greater number of corporate hospitals get sanction for transplant surgeries, the organ donation activities will increase as these hospitals stand to benefit by the surgeries occurring following the organ donation. Such hospitals set systems in place, prioritizing declaration of brain death with resultant generation of a number of organs some of which are allocated to the parent hospital as per the allocation guidelines. The other hospitals in the region would receive the rest of the organs donated by that brain-dead patient resulting in that many transplant surgeries. Thus, the facilitative effect of finance and balance sheets cannot be denied and could be used positively to bring about a rise in organ donation rates of that region.

Theme: Willingness to be a donor: Urban versus Rural

The hepatologists observed that large population of our country stays in the rural area and they have observed that the people from rural area are more willing to become donors than those living in urban areas. Alghanim in a study conducted in Saudi Arabia also had a similar observation. The study noted that people in the rural areas were more likely to become donors [36]. Hence the participants felt that it was essential to carry out awareness campaigns in the rural areas.

Theme: Incentive for donor family

The liver specialists felt that there was a need to develop a method of providing some form of incentive to the donor or in case of the brain dead or cardiac dead donor, to the family members of the donor. This may not be in form of payment, but in form of efforts to rehabilitate family members or dependents of the deceased donor. Sympathy is of no use to the dependents of the donor. The participants suggested that this incentive could be in form of providing education for dependents of the donor. However, they clearly stated that incentive should not be in form of money as that would lead to too many ethical dilemmas. Chkhotua stated that though altruism is the main principle of organ donation worldwide, it has been unable to meet the demand for organs. At the same time, it needs to be acknowledged that donors incur many expenses while participating in the transplant process, some of which are unseen as they are not billed expenses [37]. This seems unfair. It may be time to consider the various forms of incentives and discuss these from ethical, economical and legal point of view.

Conclusion

This research was aimed at studying the experiences of liver specialists from point of view of organ donation. The liver specialists suggested that people of this region lacked awareness about categories of organ donors and

about concept of brain death. They also stated that one of the challenges to organ donation in this region was lack of diagnosis of brain death by doctors of various hospitals of this region and many hospitals not having an approved brain death committee. They suggested that a common brain death committee could be created per region which could be called upon to diagnose brain death at those hospitals which did not have a brain death committee. The liver specialists also stated that there was need for more hospitals to be sanctioned for transplant surgeries. They stated that not only do these surgeries benefit patients, but the brain death declarations and the awareness campaigns run by such hospitals also benefit the region from point of view of organ donation. The liver specialists were of the opinion that there was a need to sensitize the Police personnel towards organ donation so as to prevent delay from there side for obtaining no objection certificate as well as during arrangement of the green corridor for movement of the harvested organ. They all stated that some form of non-monetary incentive had to be worked into the system to benefit the donor or the family of the donor.

Conflict of interest statement: None declared

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Original article

Health Literacy and Quality of Life of Diabetic and Non-Diabetic End Stage Renal Disease Patients

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Abstract

Introduction. Chronic kidney disease (CKD) negatively affects physical, mental and social quality of life (QoL) of patients, and requires an adequate level of health literacy (HL) for better management. End-stage renal disease (ESRD) diabetic patients have worse QoL compared to non-diabetic HD patients. This study aims to estimate the HL levels of diabetic and non-diabetic ESRD patients, also to assess the association between HL and QoL in ESRD patients.

Methods. This descriptive study of ESRD patients was conducted in dialysis units in Ankara. It included 446 patients, 223 ESRD patients with diabetes and 223 without diabetes. Patients' data were collected using face to face interviews. Test of Functional Health Literacy in Adult was used to assess HL and SF-36 to assess QoL.

Results. DM cases, verbal, numerical and general HL scores were lower than non-DM cases ($p < 0.001$). DM cases had lower scores of QoL than non-DM cases. The mean scores of physical functioning, role limitation due to emotional problems, energy, general health, physical health and mental health were found lower in cases with DM than non-DM cases ($p < 0.001$). There was a statistically positive correlation between level of HL and PHC scores ($p < 0.001$) and MHC scores ($p < 0.001$).

Conclusion. QoL and HL levels are lower in diabetic patients than non-diabetics. Effective strategies should be considered to increase QoL among ESRD patients with low levels of HL, and health professionals should be more aware of the association between HL and QoL, especially the cases with DM.

Keywords: diabetes mellitus, end stage renal disease, health literacy, quality of life

tant public health problem and it is estimated that 10% of adult population has CKD worldwide [1,2] and CKD prevalence was found to be as high as 15.7% in Turkey [3]. CKD and especially end-stage renal disease (ESRD) negatively affect the physical, mental and social QoL of patients. Good management of the disease decreases the personal burden of the disease and also increases QoL of the patients [4-7].

CKD patients having high level health literacy (HL) deal better with their disease, use healthcare services more effectively and handle their own pathology and its consequences properly [8]. HL is also very important to have better medication adherence, avoid potentially nephrotoxic substances, and avoid risky behaviors such as smoking, high dietary salt intake, and lack of exercise in CKD [9].

Diabetes mellitus (DM) is a major public health problem worldwide, patients have higher risk of life-changing and life-threatening complications. The patients with DM have higher risk of hypertension, dyslipidemia, cardiovascular complications [10]. DM also plays a major role in the pathogenesis of CKD and it is believed that DM accounts for 30-50% of all CKD cases and affects 285 million (6.4%) adults worldwide [11,12]. Although the patients with DM are expected to have higher quality of life (QoL) due to frequent use of health services, QoL in diabetic HD patients is worse than non-diabetic HD patients [13,14]. On the other hand, Bailey indicates that some studies found association between limited HL and complications in DM cases [15]. Limited HL can also have adverse effect on QoL of ESRD patients and especially the patients with DM. We aim to estimate the HL levels of diabetic and non-diabetic ESRD patients, also to assess the association between HL and QoL in ESRD patients.

Material and methods

Descriptive study of ESRD patients with and without DM was conducted in dialysis units in Ankara.

Introduction

Chronic kidney disease (CKD) is accepted as an impor-

Population and Sample Selection Criteria

G*Power (version 3.0.10) and Power Analysis and Sample Size package program were used to determine the sample size ($\alpha=0.05$ and $\beta=0.10$). Inclusion criteria were 40 years of age and over, hemodialysis duration over three months, and without immunosuppressive therapy. Gender and age groups were accepted as matching criteria to select ESRD patients with DM and without DM. Patients with communication problems, heart failure, active infection or malignancy were not included in the study.

Variables and Scales

Socio-demographic characteristics and health behaviors, history of disease, clinical and laboratory findings, health related quality of life scale (Short Form-36; SF-36) [16] and health literacy scale (Test of Functional Health Literacy in Adults; TOFHLA) [17] were included in the questionnaire of this study. SF-36, questionnaire was developed for the assessment of the health related QoL. It comprised 8 sub dimensions and physical health (PHC) and mental health (MHC) components. Turkish reliability and validity studies were done by Koçyiğit *et al.* in 1999. Cronbach alpha coefficients of each subscale and components were found to be 0.732 to 0.761 [18]. Parker *et al.* (1995) developed the TOFHLA which had verbal and numerical parts. The Turkish adaptation of this test was carried out by Üçpınar in 2014, and Cronbach alpha was 0.523 for the verbal skills, and 0.732 for the numerical skills [19].

Data Collection and Analysis

Data collection was conducted between May 12 and November 25, 2016. During the study, a total of 14 dialysis centers (four governmental and ten private), 223 DM (91 women and 132 men) and 223 non-DM (91 women and 132 men) were interviewed by two trained nurses. Statistical Package for Social Science (SPSS) 21 package program was used for the statistical analysis of the data. Chi-square test, Mann Whitney U and Spearman correlation were used in the analyses. Two logistic models were used to determine the factors affecting PHC and MHC of QoL. The quality of life (PHC and MHC) scores were divided by the median value and the backward stepwise logistic regression model were applied. PHC was cut to the median value of "45" and the MHC to the median value of "57". The reason of not using linear regression was that the data of QoL (PHC and MHC) was not normally distributed. The variables used in logistic models were age (numeric variable), gender (Ref: female), diabetes status (Ref: having DM), education status (Ref: illiterate),

hypertension status (Ref: having hypertension), coronary heart disease (Ref: having coronary heart disease), hyperlipidemia (Ref: high hyperlipidemia), CKD history in the family (Ref: positive familial CKD history), followed up in nephrology clinic (Ref: followed up in nephrology clinic), the duration of CKD (numerical), the duration of hemodialysis (numerical), known cause of the CKD (Ref: known cause) and the health literacy level (Ref: insufficient level).

This study was approved by Hacettepe University Non-Interventional Ethics Board (dated 22 March 2016 and GO16/03-07). Written permission was obtained from Ministry of Health and dialysis centers. The patients were informed before the interview, and the questionnaires were filled after signing the consent.

Results

The sociodemographic characteristics of ESRD patients with and without DM were similar in the study. There were no statistically significant differences between gender, age, educational status, marital status with and without diabetes mellitus (Table 1).

Table 1. Age, gender and educational status of ESRD patients with and without DM

		ESRD Patients				
		DM		Non-DM		p
	n	%	n	%		
Gender					1.0	
Male	132	59.2	132	59.2		
Female	91	40.8	91	40.8		
Age Group					0.200	
40-49	16	7.2	15	6.7		
50-59	45	20.2	62	27.8		
60-69	104	46.6	83	37.2		
70-79	51	22.9	52	23.4		
80 +	7	3.1	11	4.9		
Educational Status					0.096	
Illiterate	33	14.8	29	13.0		
Literate	17	7.6	7	3.1		
Primary school	92	41.3	94	42.2		
Secondary school	27	12.1	26	11.7		
High school	35	15.7	32	14.3		
University	19	8.5	35	15.7		
Total	223	100.0	223	100.0		

In non-DM cases, verbal, numerical and general HL scores were higher than DM cases ($p<0.001$). Non-DM cases had higher scores in all subscales of SF 36 scale than DM cases. The mean scores of physical functioning, role limitation due to emotional problems, energy, general health, PHC and MHC were found higher in cases without DM than DM cases ($p<0.001$). (Table 2). In the study, 76.2% of ESRD patients with DM had inadequate level of HL, this percentage was 55.2% for non-DM patients. Sufficient HL was three times more in non-DMs (17.5%) than DM (5.4%).

Table 2. Distribution of TOFLHA and SF-36 scores in ESRD patients with and without DM

	ESRD Patients				p*
	DM		Non-DM		
	Mean	SD	Mean	SD	
TOFLHA					
Verbal	23.29	10.53	28.58	11.60	<0.001
Numerical	22.04	9.95	25.96	10.57	<0.001
General	45.34	18.14	54.55	20.04	<0.001
SF-36					
Physical functioning	32.58	28.29	44.75	29.71	<0.001
Role limitation due to physical health	27.47	37.66	33.14	38.52	0.08
Role limitation due to emotional problems	60.08	43.91	70.28	41.40	0.008
Vitality	33.38	27.30	42.22	28.37	<0.001
Mental health	68.94	24.08	73.85	20.21	0.06
Social functioning	51.79	42.70	58.86	40.09	0.06
Bodily pain	67.43	33.59	69.04	31.69	0.73
General health	46.82	26.00	57.00	26.89	<0.001
Physical health compound	43.57	23.53	51.13	23.78	<0.001
Mental health compound	53.45	25.16	61.25	24.46	<0.001

*Mann-Whitney U test

In the adequate level of HL group there was no statistical significance for QoL between DM and non-DM cases. The patients without DM had significantly higher score of general health than the patients with DM in the marginal health literacy level. The patients

without DM had significantly higher scores of physical functioning, energy, general health status, and MHC than the patients with DM in inadequate health literacy level (Figure 1).

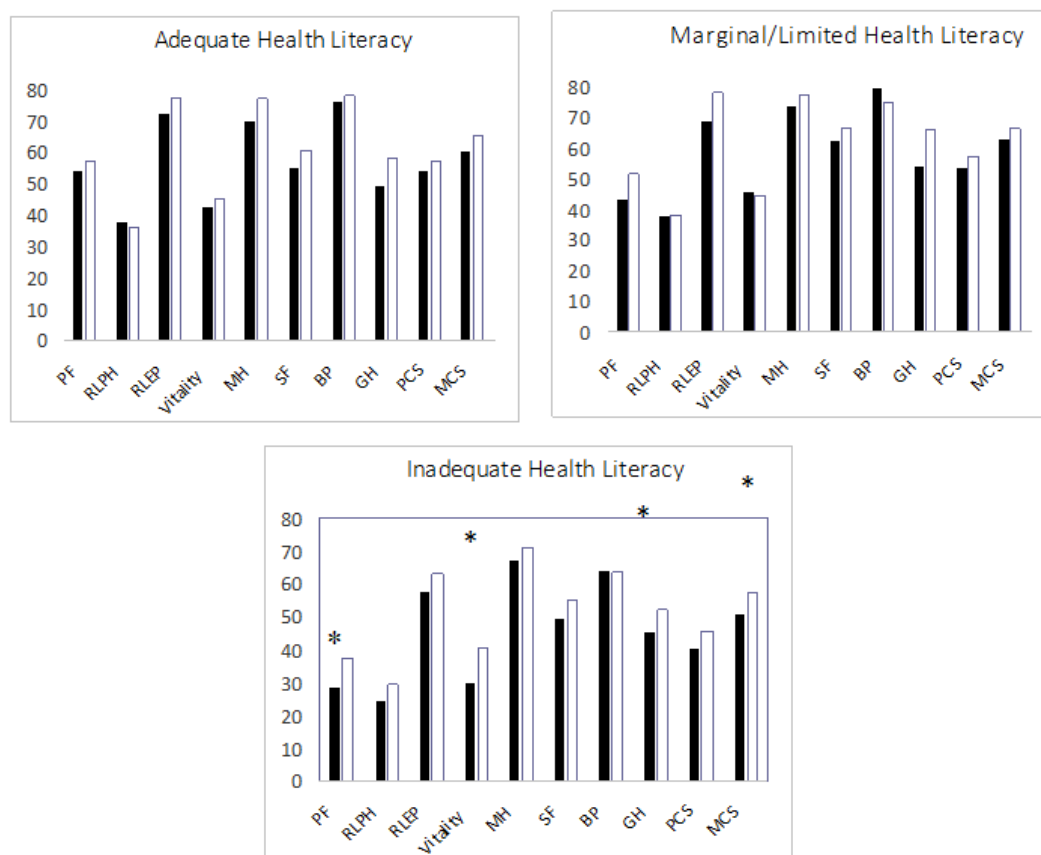


Fig. 1. The level of health literacy and QOL dimensions in DM and non-DM Patients (PF: Physical Functioning, RLPH: Role Limitation Due to Physical Health, RLEP: Role Limitation Due to Emotional Problem, MH: Mental Health, SF: Social Functioning, BP: Bodily Pain, GH: General Health, PCS: Physical Compound Summary, MCS: Mental Compound Summary, Black bars: With DM, White bars: Without DM, *Significant Result with Mann-Whitney U test)

PHC of quality of life was 3.979 times higher in males, it was decreasing with age (0.967); and also 1.761 times higher in patients without CVDs, 2.299 times higher in high school and upper educated patients. MHC of QoL was 2.058 times higher in males, 1.562 times higher in

the patients without DM, 1.582 times higher in the patients with having CKD family history (Table 3). There was a statistically significant positive correlation between level of HL and PHC scores ($r=0.311$ $p<0.001$) and MHC scores ($r=0.263$, $p<0.001$), but it was not very strong (Figure 2).

Table 3. Logistic regression models for physical and mental compound summaries of QOL

	p	Exp (β)	95% Confidence Interval	
Physical Health Compound				
Male	0.000	3.979	2.445	6.475
Age	0.004	0.967	0.945	0.989
Not having cardiovascular diseases	0.012	1.761	1.131	2.741
Education status (ref: illiterate)	0.001			
Graduated from primary school	0.732	0.900	0.494	1.640
Graduated from high school and over	0.021	2.299	1.132	4.670
Constant	0.332	2.291		
Mental Health Compound				
Male	0.000	2.058	1.394	3.038
DM Status	0.022	1.562	1.065	2.291
Not having relatives with cardiovascular diseases	0.059	1.582	0.983	2.547
Constant	0.000	0.376		

Physical Health Compound: $R^2=0.168$ (Cox ve Snell), 0.225 (Nagelkerke), 0.038 (Hosmer ve Lemeshow Test) **Mental Health Compound:** $R^2=0.050$ (Cox ve Snell), 0.067 (Nagelkerke), 0.661 (Hosmer ve Lemeshow Test)

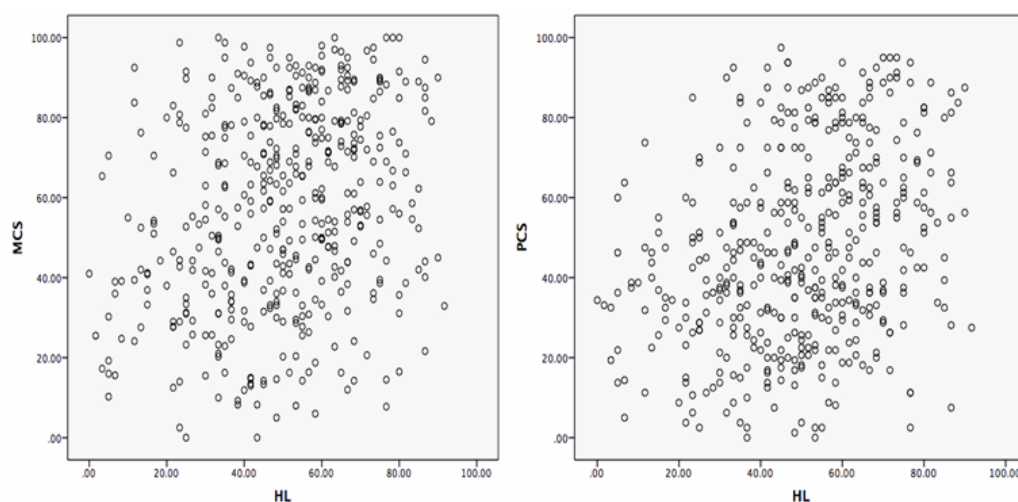


Fig. 2. The correlation between physical and mental health components and HL

Discussion

This study was conducted in total of 446 ESRD cases to estimate the effect of health literacy on quality of life and the difference between diabetic patients with DM and non-diabetic patients in four government and ten private dialysis centers located in Ankara city. People with low levels of HL have poorer chronic disease management, which increases mortality in ESRD [9, 20]. Tylor *et al.* evaluated 12,324 patients in 20 studies, but they were not all hemodialysis patients [8]. They found the median prevalence of limited HL level as 23% in all patients, and 27% (CI 19%-35%) in patients on dialysis. In the review of Taylor *et al.*, the prevalence of limited HL was found to be between

8.4-49% in five studies that used STOFHLA and there was no significance between group heterogeneity in these studies. In our study, the level of inadequate HL was higher in patients with DM (76.2%) compared to patients without DM (55.2%), but it was higher from all of the mentioned studies in the review conducted by Tylor *et al* [8]. Lai *et al.* also indicated that ESRD with DM need more health information to improve self-management, especially improve their communicative and critical HL as well as functional HL [21]. Bailey *et al.* also declared there was association between limited HL and adverse outcomes in DM cases [15]. DM patients with limited HL would have more probability of being ESRD because of insufficient self-management skills.

In this study, scores of general, PHC and MHC in SF-36 were found higher in non-DM cases than DM cases, physical functioning meaning limitation in self-care activities, role-emotional meaning having problems with daily activities as a result of emotional problems and vitality meaning feeling tired, were statically higher in non-DM cases. Gumprecht *et al.* [13] and Soleymanian *et al.* [14] presented the same findings in their studies. Hortemo *et al.* indicated that having ESRD as a long-term complication or comorbidity contribute for a decrease QoL level in DM patients [22]. Soleymanian *et al.* also indicated that the underlying comorbidities can independently and significantly affect the QoL in hemodialysis patients with DM [14].

The sociodemographic characteristics of the patients significantly affect the level of PHC and MHC of QoL in our study. Age is an important determinant for progress of the disease. Increasing age significantly decreased PHC and MHC of QoL; which is in line with the studies of Bayoumi *et al.* [23], Mcadams-Demargo *at al.* [24] and Seica *et al.* [25]. Higher level of education also increased significantly QoL in our study similar to the other studies [23,24]. Educational level could be accepted as a socioeconomic determinant, higher educated people could reach and use health care services more effectively than lower educated people; also educational level is an important determinant of HL. We also found male patients had higher level of PHC and MHC of QoL than women; The studies conducted by Mcadams-Demargo *at al.* [24] and Seica *et al.* [25] showed that male patients had higher QoL; but Bayoumi *et al.* found differently, female patients had higher QoL [23]. The review of Cobo *et al.* emphasized that gender differences have resulted in the pathogenesis of disease and new developed treatment opportunities [26]. Gender inequality or cultural determinants can play a role to decrease quality of life in women in our society. Having CVD is affecting PHC of QoL and having a relative with CVD as a risk factor of MHC of QoL in our study. Studies confirmed the significance of CKD on the development of CVD, complications or comorbidity with ESRD decreased QoL or healthy lifestyle behaviors [27]. We found correlation between HL and QoL in our study. Health literacy, which is important both in health care and personal care, affects QoL in ESRD patients. This can be regarded as a supporting finding that HL is the factor affecting QoL, which is the basic hypothesis of the research. In our logistic models, health literacy is not found as an explanatory variable, low HL in general may have led education to become more prominent. Couture *et al.* found no relationship between HL and QoL in hemodialysis cases [28]. However, the relation between HL and QoL was found in patients with hypertension by Wang *et al.* [29], in older patients with long term illness by Panagioti *et al.* [30], in breast, lung, prostate, and colorectal cancer patients by Halverson *et al.* [31], and in the patients with ischemic heart diseases by

Alejandro Gonzales-Chica *et al.* [34]. We think that it is necessary to investigate the association between HL and QoL by using quantitative studies to explain the effect of sociodemographic and cultural factors on HL and QoL.

There are also some limitations in our study. Information about the patients' illness during the communication between the physician and the patient, lifestyle changes and applications to the health institutions during the treatment process are expected to positively affect the QoL and HL. Information on the physician-patient communication process were not included in our study. Since the patients do not always go to the same hospital for their treatment, we could not use the patient data to reach the detailed progress from hospitals' surveillance systems.

Conclusion

Diabetic cases had lower levels of both QoL and HL than non-diabetic patients. Considering the increased burden and complications of CKD, suitable initiatives are required to increase health literacy especially in diabetic patients. Effective strategies should be considered to increase in QoL among ESRD patients with low levels of HL, and health professionals should be more aware of the association between HL and QoL, routine check list should be implemented into assessment procedures for ESRD patients. The evaluation of QoL of patients with the qualitative research in relation with the level of HL will shed light on the future interventions.

Conflict of interest statement: None declared

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Original article

Comparing the Level of Immunoglobulins in Patients with Primary Membranous Nephropathy under Standard Treatment and Rituximab

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Abstract

Introduction. Primary membranous nephropathy (PMN) is an autoimmune disease affecting renal glomeruli, characterized by aggregation of autoantibodies on podocytes and subsequent epithelial thickening. Rituximab (RTX), an anti-CD20 monoclonal antibody, is used to treat steroid-resistant PMN cases. Accordingly, presented study compared peripheral blood IgA, IgM, and IgG as well as IgG subclasses levels in patients under standard treatment and under RTX treatment before and after RTX administration.

Method. With method of nephelometry, 25 PMN patients, 15 under standard treatment and 10 receiving standard+RTX treatment were enrolled and compared with 15 healthy matched individuals. The RTX group was studied before (pre-RTX) and 2 months after (post-RTX) drug infusion.

Results. Serum IgG level of Std-treatment patients and healthy controls was higher compared to pre-RTX group (0.001 and 0.008 respectively). Serum total IgA and IgM levels were not statistically different between Std-treatment, pre-RTX, and healthy controls; however, IgA decreased after administration of rituximab ($P=0.015$). IgG1 and IgG2 levels in pre-RTX group was considerably lower than in patients on Std-treatment ($P=0.001$ and 0.005 , respectively) and healthy controls ($P=0.041$ and 0.019 , respectively) but were not affected by RTX. Serum IgG4 was elevated in both Std-treatment and pre-RTX groups compared to healthy controls ($P<0.0001$ and 0.010 , respectively) and decreased significantly following RTX administration.

Conclusion. Serum IgG4 has a significant prognostic value in PMN. IgG1 and IgG2 were lower in steroid-resistant cases. IgM and IgA do not appear to be invol-

ved in pathogenesis of PMN. Moreover, IgG1, IgG2 and IgM amounts were not affected by RTX.

Keywords: glomerulonephritis, membranous nephropathy, Immunoglobulin, Rituximab

Introduction

Primary (or idiopathic) membranous nephropathy (PMN) is considered the most common cause of nephrotic syndrome (NS) in adults, characterized by proteinuria, edema, hypoalbuminemia, hyperlipidemia and usually detectable circulating autoantibodies [1,2]. Histopathological features of PMN consist of subepithelial immune complex deposition and subsequent thickening of glomerular basement membrane; in fact, diagnosis of primary membranous nephropathy could merely be confirmed by ruling out secondary causes such as autoimmunity (e.g. lupus erythematosus), chronic infections (e.g. HBV, HCV, H.Pylori), drugs (e.g. gold, penicillamine) and malignancies (e.g. renal, prostate), situations which are accompanied by excessive antibody production [3,4]. Although the underlying causes of PMN have not yet been completely discovered, recent studies have introduced some autoantigens on podocytes which are targeted by autoantibodies detectable in blood circulation of most PMN patients [5]. The most known autoantigen herein is phospholipase A2 receptor (PLA2R), a member of the mannose receptor family. The specificity of anti-PLA2R antibody for diagnosis of PMN has been estimated at 70-80% whereas it is undetectable in healthy individuals. Evaluation of anti-PLA2R antibodies is performed for both diagnosis and follow up purposes in clinical field as its presence and persistence is generally accompanied by worse prog-

nosis [6]. The other autoantigen targeted by autoantibodies in PMN is thrombospondin type1 domain-containing 7A (THSD7A), membrane-associated N-glycoprotein which mediates endothelial cell migration and tube formation, found in almost 5% of PMN cases [2]. Lecithin-cholesterol acetyl transferase (LCAT), an enzyme involved in maintaining cholesterol homeostasis, has recently been suggested as another probable target [7]. Of note, in contrast to the secondary membranous nephropathies which could be accompanied by increased level of IgG1, IgG2, and IgG3, the prominent autoantibody isotype directed against abovementioned antigens in PMN is IgG4 [4].

In addition to the supportive care including blood pressure control, angiotensin blockers to minimize proteinuria, statins for treating hyperlipidemia, low protein diet, salt restriction and diuretics to control edema, patients with progressive decrease of glomerular filtration rate (GFR) or refractory proteinuria should undergo immunosuppressive therapy by drugs such as steroids, cyclophosphamide, calcineurin inhibitors (cyclosporine, tacrolimus), and finally rituximab (RTX) [8]. RTX has been shown to ameliorate refractory form of PMN demonstrated by reducing urine proteins following administration [9]. RTX a chimeric monoclonal anti-

CD20 antibody eliminates autoantibody-producing B cells by direct signaling, complement-mediated cytotoxicity (CMC), and antibody-dependent cellular cytotoxicity (ADCC) [10]. It might also bind directly to the sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL-3b) on podocytes protecting these cells from their disruption [11,12]. Regarding the considerable role of antibodies in pathogenesis of PMN, we aimed to evaluate the level of various antibody subtypes and subclasses in steroid-responsive and refractory PMN patients (before and after receiving RTX) in order to study their predictive and pathologic value.

Material and methods

Patients

Patients were enrolled according to the clinical, laboratory and pathological diagnostic criteria of primary membranous nephropathy, including those who were under standard treatment (steroid and immunosuppressive drugs, shown in table 1) and refractory cases who received standard treatment plus rituximab [13]; the latter group was studied just before administrating rituximab (Pre-RTX) and two months afterwards (Post-RTX).

Table 1. Demographic and laboratory data of studied population (at sampling time)

Group	Healthy control	Post-Rituximab	Pre-Rituximab	Standard treatment	P value
<i>N</i>	15	10	10	15	
Age in years: mean \pm SEM	45.8 \pm 3.5	52.2 \pm 3.49	52.2 \pm 3.49	48 \pm 3.26	0.56
Gender ratio (M:F)	8: 2	8: 2	8: 2	11: 4	
Creatinine: Mean \pm SEM	1 \pm 0.03	1.44 \pm 0.1	1.81 \pm 0.1	1.25 \pm 0.03	<0.0001
BUN: Mean \pm SEM	19.2 \pm 0.93	36.8 \pm 1.36	50.4 \pm 2.26	36.9 \pm 1.74	<0.0001
WBC: Mean \pm SEM	5020 \pm 270	4342 \pm 341	6890 \pm 261	6620 \pm 261	<0.0001
Total lymphocyte percent	42.3 \pm 1.47	33.5 \pm 1.5	54.1 \pm 1.98	48 \pm 2	<0.0001
Urine protein (mg/dl)	0: 15	<300: 2 <3000: 8	3000-4000: 5 4000-5000: 3 >5000: 2	500-800: 12 1000-1500: 1 1500-3000: 1 >3000:1	
Anti- PLA2R	-	Positive: 1 Negative: 9	Positive: 3 Negative: 7	Negative: 15	
<i>IS protocol:</i>					
Pred, Cyclosporin Pred,		7	7	12	
Cyclosporin, Mycophenolate	-	0	0	1	
Pred, Tacrolimus		2	2	2	
Pred, Mycophenolate, Tac		1	1	0	

Standard treatment: PMN patients under standard treatment (steroid + immunosuppressive); Pre-Rituximab: PMN patients under standard treatment + Rituximab before receiving the drug; Post-Rituximab: PMN patients under standard treatment + Rituximab two months after administration; WBC: white blood cells; IS: immunosuppression; PLA2R: phospholipase A2 receptor; Pred: prednisone; SEM: standard error of mean

Serum isolation and measurement of immunoglobulins

Serum samples were collected in serum separating tubes and were separated immediately using centrifuge. Then they were aliquoted and stored in freezers at minus 70 degrees until the end of sampling period. Immunoglobulins level were measured using nephelometry

technique, (MininephTM Human Ig Kit, The Binding Site Ltd., Birmingham, UK).

Statistical analysis

Data were presented as mean \pm SEM (standard error of the mean). The comparison between groups was per-

med by non-parametric Kruskal-wallis test. Comparison between pre and post RTX values was performed by non-parametric Wilcoxon test (SPSS 22; SPSS Inc., Chicago, USA). P values less than 0.05 were considered as statistically significant.

Results

The demographic and laboratory data of patients and healthy controls are summarized in table 1.

Table 2. Immunoglobulins levels in studied groups (mg/dl)

Group	IgG (mean±SEM)	IgA (mean±SEM)	IgM (mean±SEM)	IgG1 (mean±SEM)	IgG2 (mean±SEM)	IgG4 (mean±SEM)
Std-treatment	1167±60	180±4	141±11	615±44	417±24	53±5
Pre RTX	812±60	175±14	130±14	381±38	279±28	40±4
post RTX	638±50	122±13	123±10	344±33	198±32	25±5
Healthy controls	1038±50	152±17	125±9	483±36	389±20	24±2

Std: standard; RTX: Rituximab; SEM: standard error of mean

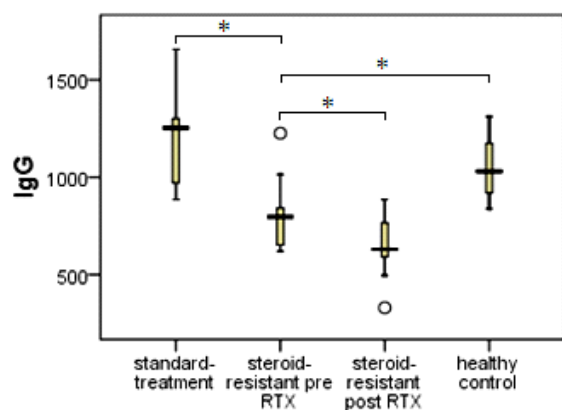
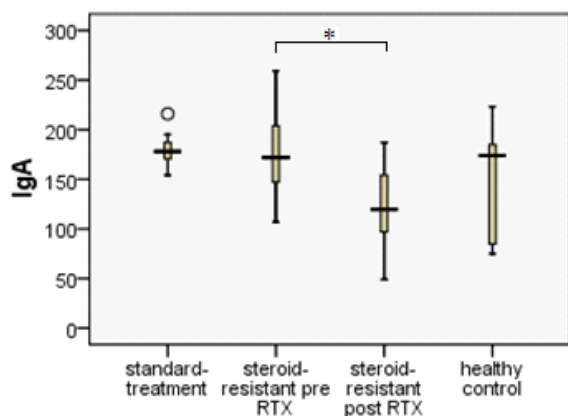


Fig. 1. Serum IgG levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

Serum total IgA levels were not statistically different between Std-treatment, pre-RTX, and healthy control groups; however, they were reduced after administration of RTX ($P=0.015$) (Figure 2). Serum total IgM levels were almost similar in all studied groups and were not affected by RTX therapy (Figure 3).



Antibody subtypes difference between groups

Serum levels of IgG, IgA and IgM as well as IgG subclasses IgG1, 2 and 4 are shown on Table 2. Serum total IgG level in standard-treatment patients were higher than in the pre-RTX group (0.001) but almost similar to healthy controls. IgG levels of pre-RTX patients were significantly lower than in healthy controls ($p=0.008$) and decreased further after RTX administration ($p=0.035$) (Figure 1).

Fig. 2. Serum IgA level in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

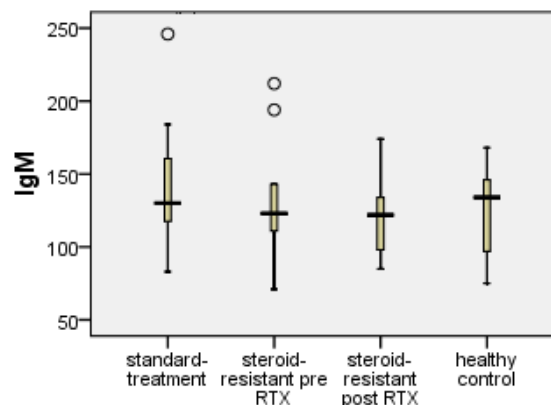


Fig. 3. Serum IgM level in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups

IgG subclasses differences between groups

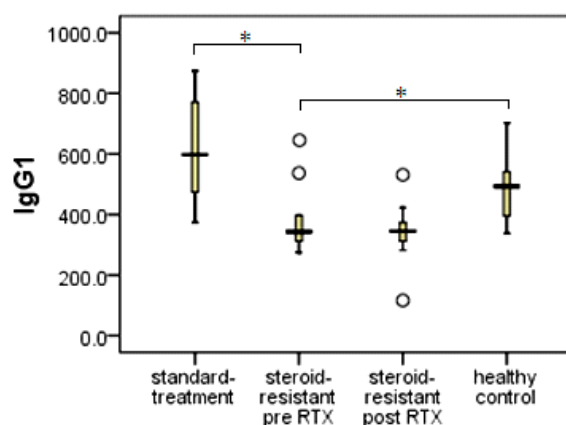


Fig. 4. Serum IgG1 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

IgG1 levels pre-RTX were considerably lower than in patients on Std-treatment ($P=0.001$) and healthy controls ($P=0.041$). Moreover, no change was observed after RTX therapy (Figure 4). Similarly, serum IgG2 levels showed significant decrease in pre-RTX group compared to the Std-treatment ($p=0.005$) and healthy controls ($p=0.019$) but they were not affected by RTX

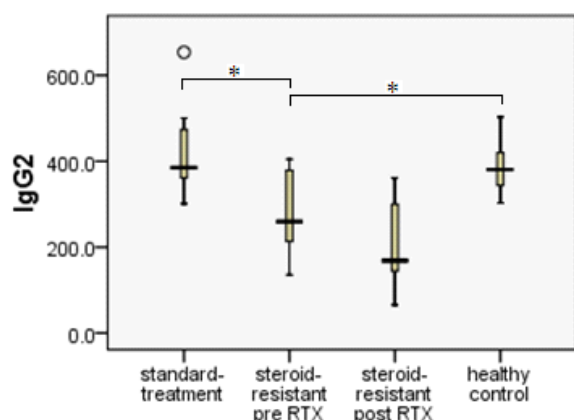


Fig. 5. Serum IgG2 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy groups (*:P.value<0.05)

(Figure 5). Serum IgG4 levels were elevated in both Std-treatment and pre-RTX groups compared to the healthy controls ($P < 0.0001$ and 0.01 , respectively) and decreased significantly after RTX administration ($P= 0.011$) (Figure 6). Comparison of immunoglobulin values is summarized in Table 3.

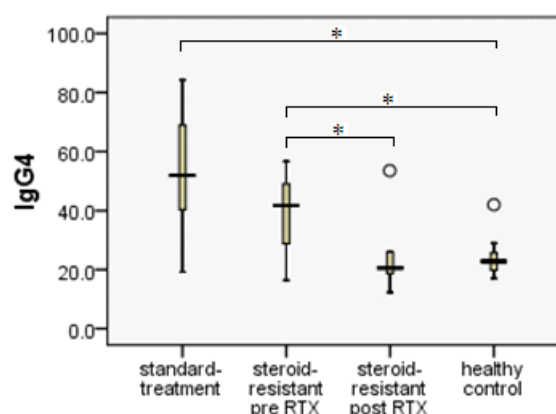


Fig. 6. Serum IgG4 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

Table 3. Comparison of immunoglobulins levels between studied groups

Compared Groups	IgG (p.value)	IgA (p.value)	IgM (p.value)	IgG1 (p.value)	IgG2 (p.value)	IgG4 (p.value)
Std-treatment/pre Rtx	0.001*	0.598	0.523	0.001*	0.005*	0.56
Std-treatment/healthy control	0.202	0.36	0.405	0.067	0.618	<0.000*
Pre RTX /healthy control	0.008*	0.496	0.853	0.041*	0.019*	0.010*
Pre RTX /post RTX	0.035*	0.015*	0.796	1	0.063	0.011*

Std: standard; RTX: Rituximab

Discussion

Recent studies have shown that in most patients with primary membranous nephropathy anti-PLA2R IgG4 auto-antibodies are detectable in blood circulation whereas this is absent in secondary forms of the disease [4]. Although the significance of autoantibodies in pathogenesis of membranous nephropathy has been shown, the probable relationship between antibody level and disease stage or prognosis has not yet been clarified. Bazzi *et al.* assessed the fractional excretion of IgG (FE-IgG) in 84 patients with idiopathic membranous nephropathy and after a follow up period of 7 years they found that higher FE-IgG amounts could predict increased risk of kidney failure and lower possibility of remission. In addition, combined treatment by steroids and cyclophosphamide decreased the progression rate and increased the remission rate in patients with high FE-IgG [14]. In another study Lönnbro-Widgren compared glomerular IgG sub classes deposition between patients with PMN and those with secondary membranous nephropathy (MN) due to malignancy. There was a significant correlation between the absence of IgG4 and malignancy-related MN while the other

IgG subclasses' amount did not differ significantly between the two groups; IgG2-positivity was present in most patients in both groups but neither IgG1 nor IgG3 seemed to be implicated in the pathogenesis of membranous nephropathies [15]. Later, von Haxthausen *et al.* found no difference in level of IgG subclasses between primary and malignancy-associated MN. Additionally, higher level of these antibodies did not show any association with disease progression rate [16]. However, Na *et al.* comparing IgG subclasses in membranous lupus nephritis and idiopathic membranous nephropathy found differences between the two groups apart from IgG1 [17]. Another study revealed IgG4 and IgG1 dominance in primary and secondary membranous nephropathies respectively. Moreover, at early stages of PMN IgG1 was the most frequent subtype in tissue biopsy although by disease progression IgG4 got the first place [18]. Kuroki *et al.* has evaluated the level of glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis (DPLN), membranous LN lupus nephritis (MLN) and primary membranous nephropathy. They found IgG1, IgG2, IgG3, and at a lesser amount IgG4 in glomerular deposits of DPLN and MLN while IgG4 was the dominant glomerular IgG subclass in PMN.

The mean serum IgG subclasses concentration in DPLN and MLN were almost similar to the healthy controls, except for IgG1 which was increased in MLN. In PMN patients, the mean IgG4 was increased; however, the mean concentration of other IgG subclasses was significantly decreased [19]. Liu *et al.* showed elevated expression of anti-PLA2R antibodies and IgG4 level in PMN patients comparing to the secondary MN as well as non-MN cases. Moreover, a strong correlation was found between anti-PLA2R serum level and tissue deposition [20]. These findings were confirmed by Yeo *et al.* who calculated 83% sensitivity and 88% specificity for PLA2R, and 76% sensitivity and 86% specificity for IgG4 in diagnosis of PMN. When both were positive, the specificity raised up to the 96.4% [21].

Juozapaite *et al.* evaluated the significance of the IgM deposits in the mesangium of children with nephrotic syndrome but they did not find any significant difference in the outcome of IgM-positive and IgM-negative patients [22]; however, it was found that the circulating IgG and IgM antibodies of PMN patients showed an altered pattern of reactivity to the self-antigens compared to the healthy controls in spite of their normal reactivity to the non-self-antigens [23]. Le Viet *et al.* studied the predictive value of serum IgA, IgG and IgM in PMN children. They reported median serum levels of 1.15, 2.23, and 1.7 g/L for IgA, IgG, and IgM respectively. Serum IgA and IgG levels in patients were significantly lower than healthy controls. Moreover, IgG amount had a positive predictive value for steroid-resistant nephrotic syndrome (SRNS). With the cutoff point of 2.04 g/L, this test had the sensitivity and specificity of 89.5% and 95.5%, respectively. The IgG/IgM ratio also showed a positive predictive value for SRNS (AUC=0.892, $P < 0.001$). Therefore, serum IgG level and IgG/IgM ratio might be considered as predictive markers for steroid resistance in children with idiopathic nephritic syndrome [24]. Moreover, Branten *et al.* suggested urine IgG with sensitivity and specificity of 88% as a helpful marker in predicting renal failure in PMN patients; this could be used to make appropriate decisions on the dose and combination of immunosuppressive regime [25]. Our study also demonstrated higher serum levels of IgG4 in both steroid-responsive and refractory patients which decreased significantly following RTX administration. This finding is in concordance with most previous studies indicating the implication of IgG4 in pathogenesis of PMN and suggesting its diagnostic value. Despite elevated amount of IgG4, the IgG1 and IgG2 were found in lower amounts in steroid-resistant cases and were not affected by RTX. The decreased level of IgG1 and IgG2 in pre-RTX group might be attributed to the previous infusions of rituximab which had reduced B cells general population. IgA and IgM levels showed no significant difference between studied groups implying their irrelevance to disease.

Conclusion

In summary, evaluating the IgG4 level not only in tissue biopsies and urine but also in peripheral blood could be useful in predicting the responsiveness of PMN patients to the standard treatment. This could also be used during the follow up period of patients as elevated IgG4 levels might be suggestive of uncontrolled disease. Serum amounts of IgA, IgM, IgG1 and IgG2 do not appear to be associated with the pathogenesis of PMN whereas rituximab showed the least (or null) effect on serum IgM concentration.

Conflict of interest statement: None declared

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Original article

Interleukin-19 in Diabetic Nephropathy

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Abstract

Introduction. Proinflammatory cytokines play an important role in the establishment of arteriosclerosis and kidney injury. Inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy. IL-19 has vital functions in many inflammatory processes and also can induce the angiogenesis of endothelial cells. The aim of our study was to investigate the role of interleukin-19 in development of diabetic nephropathy.

Methods. A total number of 112 persons were included and classified into 4 main groups: group I: The control group; 28 age and sex matched subjects, group II: 28 patients with type 2 diabetes without nephropathy (normoalbuminuria), group III: 28 patients with type 2 diabetes with nephropathy (microalbuminuria) and group IV; 28 patient with type 2 diabetes with nephropathy (macroalbuminuria). All subjects were submitted to: complete blood count, complete urine analysis, fasting and random blood glucose, glycosylated haemoglobin (HbA1c), serum creatinine and urea, urinary albumin excretion rate (UAE), albumin creatinine ratio (ACR), lipid profile and serum Interleukin-19 levels assays.

Results. CRP and serum IL-19 levels were significantly higher in diabetic patients compared to controls. IL-19 levels were significantly positively correlated with serum creatinine, ACR, UAE, HbA1c and CRP. Multivariable logistic regression analysis showed that IL-19 levels were independently associated with patients with DN.

Conclusion. IL-19 levels were elevated in patients with diabetic nephropathy and were positively correlated with ACR, UAE, HbA1c and CRP. IL-19 may play an important role that contributes to the progression of diabetic nephropathy.

Keywords: interleukin-19, diabetic nephropathy

Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disease and characterized by hyperglycemia which is due to the deficiency in peripheral insulin effects (insulin resis-

tance). Macrovascular and microvascular complications are the primary causes of morbidity and mortality in diabetes. It is important to understand the risk factors in order to prevent the development and progression of such complications [1]. Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, loss of glomerular filtration rate (GFR), and arterial hypertension in diabetics [2].

The pathophysiology of diabetic nephropathy is caused by both metabolic alterations (hyperglycemia and possibly hyperlipidemia) and hemodynamic alterations (systemic and glomerular hypertension). Other factors, such as inflammation, endothelial dysfunction and oxidative stress, are also involved [3].

Inflammation plays some important roles in the pathogenesis of DN. Leukocytes, macrophages and monocytes all involve in the process of DN, and proinflammatory cytokines and inflammatory markers are strongly associated with the development of DN [4].

Interleukin (IL)-19 is a member of the IL-10 family of cytokines. Secreted IL-19 is composed of 159 amino acids that form α -helical structure. IL-19 is produced by activated monocytes, and to a lesser extent, by B cells [5]. It has been reported that IL-19 can promote the T-helper2 (Th2) response, which is associated with a wide variety of allergic conditions (i.e., asthma and atopic dermatitis), type 1 diabetes, and cardiovascular disease. IL-19 have indispensable functions in many inflammatory processes and also can induce the angiogenic potential of endothelial cells [6]. A previous study also reported that IL-19 is closely related to T2DM with vascular complications. However, whether there are some association between IL-19 concentration and DN have not been revealed clearly yet [7].

Material and methods

This is a case-control study that included 112 subjects after their written and informed consent. The study was cleared by the institutional ethics committee on human research and has been conducted in the departments of Internal Medicine and Clinical Pathology, Faculty of

Medicine, Zagazig University, from September 2017 to August 2018.

Participants and groups

A total number of 112 persons were included and classified into 4 main groups: group I: The control group; age and sex matched subjects included 16 males (57.1%) and 12 females (42.9%), group II: patients with type 2 diabetes without nephropathy (normo-albuminuria). It included 16 males (57.1%) and 12 females (42.9%), group III: patients with type 2 diabetes with nephropathy (microalbuminuria); It included 12 males (42.9%) and 16 females (57.1%), and group IV: patient with type 2 diabetes with nephropathy (macroalbuminuria); It included 12 males (42.9%) and 16 females (57.1%). **Inclusion criteria:** Co-operative patients and both sexes with type 2 diabetes mellitus (T2DM) were eligible. **Exclusion Criteria:** patients with type 1 diabetes, patients with confounding factors for proteinuria and those previously diagnosed with urolithiasis, recent or current viral hepatitis or cirrhosis of liver, medical history of clinical cardiovascular disease, chronic lung disease, acute or chronic infections, with autoimmune disorders or with malignancy and pregnant or lactating females.

Physical examination and measurements:

All subjects of the study were submitted to: 1-Full history taking and thorough physical examination. Fundus examination was performed to confirm diabetic retinopathy in participants with albuminuria to confirm the diagnosis of DN.

2-Investigations (to verify the inclusion and exclusion criteria of studied subjects) including: A) Routine investigations: complete blood count ((by Sysmex KX21N), Fasting and random blood glucose, glycosylated haemoglobin (HbA1c), serum creatinine and urea, liver function tests, lipid profile and C-reactive protein (CRP). Complete urine analysis by uriscane analyzer, determination of urinary albumin excretion (UAE) and creatinine then calculation of albumin creatinine ratio (ACR),

C-reactive protein (CRP) and urinary albumin were determined by Immunoturbidimetric assay, these parameters were measured by Cobas 8000 (Roche diagnostics). B) Special investigation including: Serum Interleukin-19 levels determined by double antibody sandwich enzyme linked immunosorbent assay (ELISA), Kit provided by Glory Science Co., Ltd. (2400Veterans Blvd. Suite16-101, Del Rio, Tx 78840, USA).

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 24.0 for windows (SPSS Inc., Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test (χ^2) and Fisher exact was used to calculate difference between qualitative variables as indicated. Quantitative data were expressed as mean \pm SD (Standard deviation). One-way ANOVA test was used to compare between more than two dependent groups of normally distributed variables while Friedman's test ranks test was used for non-normally distributed variables. Pearson's and Spearman's correlation coefficient were used for correlating normal and non-parametric variables respectively. We considered values near to 1 as strong correlation & values near 0 as weak correlation. Regression analysis using the stepwise method was used to determine the association between IL-19 and diabetic nephropathy. All statistical comparisons were two tailed with significance level of P-value ≤ 0.05 indicating significant, $p < 0.001$ indicating highly significant difference while, $P > 0.05$ indicating Non-significant difference.

Results

There is a high statistically significant difference among the four studied groups regarding body mass index (BMI) and systolic blood pressure (SBP) (Table 1). A high statistically significant difference is found among the four studied groups regarding fasting blood glucose

Table 1. Demographic data of the four studied groups

Groups Variables		Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F / χ^2	P
Age (years)							
Mean \pm SD		53.43 \pm 5.12	60.04 \pm 13.83	58.07 \pm 13.83	57.71 \pm 12.07	1.564	0.202
Sex	Male: N(%)	16 (57.1%)	16 (57.1%)	12 (42.9%)	12 (42.9%)	2.294	0.514
	Female:N(%)	12 (42.9%)	12 (42.9%)	16 (57.1%)	16 (57.1%)		
BMI (kg/m ²)							
Mean \pm SD		24.36 \pm 1.32	27.88 \pm 1.43	26.82 \pm 1.69	29.12 \pm 1.46	25.698	<0.001
SBP							
Mean \pm SD (mm Hg)		113.57 \pm 6.22	119.64 \pm 13.39	123.75 \pm 14.95	118.75 \pm 11.59	3.402	0.020
DBP							
Mean \pm SD (mm Hg)		74.64 \pm 5.59	76.07 \pm 6.85	76.77 \pm 8.19	75.36 \pm 11.29	0.349	0.790

BMI: Body mass index, **SBP:** Systolic blood pressure, **DBP:** Diastolic blood pressure. p-value<0.05 is significant

Table 2. Comparison of different variables among the four studied groups

Groups Variables	Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F	P
FBG(mg/dL)						
Mean ± SD	79.89±13.51	131.36±56.65	141.82±73.37	150.89±94.83	6.354	0.001
RBG(mg/dL)						
Mean ± SD	92.07±8.23	160.93±93.86	194.64±98.85	180.14±66.26	10.020	<0.001
HbA1c (%)						
Mean ± SD	5.19±0.335	7.77±1.74	8.64±1.49	8.49±1.18	18.428	<0.001
Duration of DM						
Mean ± SD (years)	-----	8.73±4.73	15.26±4.01	19.21±4.86	16.525	<0.001
S. Cr(mg/dL)						
Mean ± SD	0.882±0.136	1.13±0.533	3.48±1.16	5.45±2.91	45.749	<0.001
ACR(mg/g)						
Mean ± SD	18.54±4.39	25.82±4.26	39.79±4.4	64.46±2.52	76.012	<0.001
UAE (mg/24h)						
Mean ± SD	9.62±2.15	22.18±1.62	280.18±93.16	532.83±180.34	38.629	<0.001
TC (mg/dL)						
Mean ± SD	163.32±34.91	186.71±25.48	201.12±67.85	218.50±54.17	6.490	<0.001
TG (mg/dL)						
Mean ± SD	162.11±46.39	176.29±23.88	210.71±61.29	211.39±56.33	7.167	<0.001

FBG: Fasting blood glucose RBG: Random blood glucose HbA1c: HemoglobinA1C, DM: diabetes mellitus S.Cr: Serum Creatinine, ACR: Albumin/creatinine ratio, UAE: urinary albumin excretion,, TC: Total cholesterol, TG: Total triglycerides, p-value<0.05 is significant

Table 3. Comparison of different Inflammatory markers of the studied groups

Groups Variables	Group I (N=28)	Group II (N=28)	Group III (N=28)	Group IV (N=28)	F	P
CRP (mg/L)						
Mean ± SD	0.761±0.179	8.2±4.42	19.64±6.71	23.25±9.97	73.318	<0.001
IL-19 (pg/ml)						
Mean ± SD	224.36±139.61	240.32±178.43	1013.57±1202.08	1457.64±1387.71	12.058	<0.001

CRP: C-reactive protein, IL-19: Interleukin-19, p-value<0.05 is significant

se (FBG), random blood glucose (RBG), HbA1c, DM duration, serum creatinine, albumin creatinine ratio (ACR) and urinary albumin excretion (UAE), total cholesterol (TC) and triglycerides (TG) (Table 2). There is a high statistically significant difference among the four studied groups regarding CRP and serum IL-19 where they increased in diabetic patients (groups II, III, and IV) compared to controls (group I) (Table 3). A significant positive correlation is found between IL-19 and BMI, DM duration, serum creatinine, ACR, UAE, FBG, HbA1c, CRP, TC and TG (Table 4). Multivariable logistic regression analysis shows that IL-19 levels are independently associated with patients with DN (Table 5).

Table 4. Correlation between IL-19 and other variables in all patients' groups

IL-19 Variables	r	p
Age	0.040	0.672
Body mass index	0.213	0.024
DM duration	0.447	<0.001
Serum Creatinine	0.414	<0.001
Albumin/creatinine ratio	0.505	<0.001
Urinary albumin excretion	0.498	<0.001
Fasting blood glucose	0.323	0.001
HbA1c	0.375	0.001
C-reactive protein	0.467	<0.001
Total cholesterol	0.291	0.002
Total triglycerides	0.334	<0.001

P-value<0.05 is significant

Table 5. Multivariable logistic regression analysis, to detect the association between IL-19 levels and diabetic nephropathy

	β	S.E.	Wald	P-value	B	95% C.I. for B	
						Lower	Upper
IL-19	0.005	0.001	21.267	<0.001	1.005	1.003	1.007
Constant	-2.123						

P-value<0.05 is significant

Discussion

Diabetic nephropathy is a major microvascular complication of diabetes mellitus (DM), it is the leading cause of end-stage renal disease. In type 1 diabetes, it deve-

lop in about 20%-30% of patients, whereas it occurs in about 10%-20% of those with type 2 diabetes [8].

Pathogenesis of DN is multifactorial element, including genetic and environmental factors, which trigger more complex pathological processes [9]. Intensive research

on molecular and cellular aspects demonstrated that immunological and inflammatory factors play essential roles in DN and its progression [10].

Inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy [11]. However, the role of inflammatory cytokines in development and progression of DN is still lacking. Extending the knowledge regarding the role of inflammation in the development and progression of DN is useful to find novel therapeutic strategies. In line with this, our study aimed to investigate the role of interleukin-19 in diabetic nephropathy and its association with DN.

Our findings showed that there was a highly significant difference among the studied groups regarding BMI, where was higher in albuminuria patients than normoalbuminuric patients. Another study found that obesity-associated glomerular hyperfiltration, renal vasodilation, increases in the glomerular filtration rate and intraglomerular capillary pressure, and increased blood pressure also are characteristics of diabetic nephropathy [12].

Also, there was a highly significant difference among the studied groups regarding SBP, it was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. In agreement with another study that suggested that microalbuminuria precedes hypertension more commonly in DM1 than DM2 [13]. A highly significant difference was found among the studied groups regarding DM duration which was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. Also, a previous study reported that a long duration of diabetes and poor glycaemic control is associated with increased production of glycosylation end products, metabolic derangements, endothelial injury, and oxidative products [14].

Regarding serum creatinine there was a highly significant difference among the four studied groups which was higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. In agreement with another study which reviewed aspects of the association of diabetes with renal disease, emphasizing that CKD and albuminuria are associated with increased rates of cardiovascular disease (CVD) and mortality [15].

Among the four studied groups regarding serum triglycerides (TG) and total cholesterol (TC), there were again highly significant differences, which were higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric patients. Also, a previous study stated that DN is associated with an altered lipid profile characterized by elevated triglyceride rich lipoproteins even in the early stages of the renal disease [16].

In the current study, fasting blood glucose (FBG), random blood glucose (RBG), HbA1c, albumin creatinine ratio (ACR) and urinary albumin excretion (UAE) in the microalbuminuric and macroalbuminuric diabetic group were significantly increased compared to normo-

albuminuric and control groups. This study is in agreement with a previous study, which has suggested that hyperglycemia is the driving force for the development of DN [17].

Positive correlation was seen between IL-19 and BMI, DM duration, serum creatinine, ACR, UAE, FBG, HbA1c, CRP, TC and TG. These results may suggest that long term hyperglycemia could increase the expression of IL-19 via stimulating endothelial cells, which results in local inflammation and accelerate endothelial damage and atherosclerosis. On the same hand, another study demonstrated a positive correlation between IL-19 and HbA1c and UAE [7].

Findings from the study also showed highly significant difference regarding C-reactive protein among studied groups, which were higher in macroalbuminuric and microalbuminuric patients than in normoalbuminuric patients. C-reactive protein, a marker of inflammation, has been reported to be associated with the risk of DM complications [17]. This result is consistent with a previous study that showed that CRP may deteriorate the inflammatory cascade in tissue injury in addition to initiating endothelial damage and atherosclerosis [18].

IL-19 concentrations were significantly higher in macroalbuminuric and microalbuminuric patients than normoalbuminuric patients. Similarly, a previous study [23] reported that inflammatory cytokines and inflammatory stimuli can prompted IL-19 to express, the expression of IL-19 is ascribed in injured and stimulated vascular smooth muscle cells [19]. Also, another study has revealed that the roles of IL-19 in development of vascular inflammatory diseases such as atherosclerosis, restenosis, and coronary artery transplant vasculopathy [8]. Similar observation was reported by another study which documented that chronic inflammation, characterized by elevated circulating levels of inflammatory markers, appears to play a critical role in the pathogenesis of T2DM and its associated complications [20].

On the same hand, a previous study reported that proinflammatory cytokines play an important role in the establishment of arteriolosclerosis and kidney injury and inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy [11].

Multivariable logistic regression analysis showed IL-19 levels were independently associated with DN which is similar to another study which reported the same results [7]. These results suggest that IL-19 involved in the inflammatory reaction and plays a significant role in the progression of DN.

Conclusion

The previous findings of this study showed that IL-19 levels were significantly high in patients with diabetic nephropathy and were associated CRP, ACR, UAE and HbA1c. The results suggest that IL-19 has a possible

role in the pathophysiology and progression of DN, providing further concepts as a therapeutic target for prevention or delaying progression of DN.

Conflict of interest statement: None declared.

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Original article

Importance of Vascular and Valve Calcifications in Chronic Kidney Disease

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Abstract

Introduction. Cardiovascular diseases (CVD) are a leading cause of increased mortality in patients with chronic kidney disease (CKD). Vascular and valve calcifications are a consequence of the mineral-bone disorders and one of the causes of CVD progression. We compared the calcium burden on arterial vessels and heart's aortic and mitral valves with clinical and laboratory data in patients with CKD.

Methods. The study included 47 patients (35 males, 12 females) with glomerular filtration rate below 44 ml/min/1.73 m². Calcium score was determined after performing abdominal laterography (individual aortic segments were analyzed at L1-L4 levels of lumbar vertebra). Pelvic and hand radiography with calcium assessment on arterial vessels and total calcium score was stated. The heart ejection fraction and the aortic/mitral valves calcium score were determined by echocardiography.

Results. The results of the clinical study showed that higher arterial calcium score correlated with: greater aortic and mitral valve calcium load, defined a lower heart ejection fraction and increased morbidity of atrial fibrillation and ischemic heart disease. Higher calcium score from abdominal aorta was associated with upper normal level and elevated serum alkaline phosphatase values ($p < 0.05$).

Conclusion. Echocardiography and X-ray allow accurate and quantitative determination of vascular and valve calcifications in patients with CKD, which compared with clinical and laboratory data can be used as a method of assessing the risk of cardiovascular morbidity and mortality in patients with CKD.

Keywords: cardiovascular disease, chronic kidney disease, valve calcifications, vascular calcifications

Introduction

Cardiovascular diseases are leading cause of increased

mortality in patients with chronic kidney disease (CKD). Vascular and valve calcifications as a consequence of mineral-bone disorders are one of the potential mechanisms for the progression of cardiovascular diseases, which are observed early in the course of CKD [1-4]. Arterial calcifications (AC) are developed in the intima and media of the medium size arteries. Medial calcification is more commonly seen in patients with diabetes mellitus and CKD [5-8] and is described by Mönkeberg calcification [8-10]. Myocardial infarction from stenosis, ischemia or acute thrombosis may be a consequence of intimal calcification in atherosclerotic disease. Arterial rigidity resulting in decreased elasticity of the vessel wall and inadequate vasodilation under conditions of increased stress is caused by calcification of the media. Theoretically, these processes can develop in the coronary arteries and lead to arrhythmias and sudden death [11]. Valve calcifications (VC), although they are less common pathology, have the same risk factors and pathogenic mechanisms as AC [12]. Poor prognosis is associated with the formation of mitral and aortic valve calcifications, even in the absence of hemodynamically significant stenosis, both in the general population [13] and in patients with CKD [14]. The purpose of this study is to compare the calcium burden determined by radiography and echocardiography on arterial vessels and heart's aortic valve (AoV) and mitral valve (MV) with clinical and laboratory data in patients who have different stages of CKD.

Material and methods

We observed 47 patients (35 males, 12 females) who had different stages of CKD (glomerular filtration rate below 44 ml/min/1.73 m², using MDRD formula). Total vascular calcium score was determined using classical abdominal aorta laterography at the lumbar vertebrae levels (L1-L4) according to the method of L. I. Kauppila *et al.* [15] and X-ray of the radial arteries and their branching to the first finger, two internal iliac and femoral arteries-a method proposed by T. Adragao *et al.* [16]. The assessment of abdominal aorta calcium

score was formed by the grade of involvement of each segment of the anterior and posterior wall of the vessel along the first four lumbar vertebrae. Calcification affecting less than 1/3 of the anterior wall of the aorta along the lumbar vertebral body received 1 score and calcification extending over 1/2 of the vertebral body length received 3 scores (total score-24) [9,15-17]. The method of Adragao *et al.* [16] was defined as for each artery; iliac, femoral, radial and first finger on the hands that was affected by calcifications. One point was given for single affected artery, the total maximum score being 8 points for all of the examined arteries [16]. The total vascular score is the sum of all arteries calcium load (maximum value-32). The calcium score of AoV and MV was formed by giving 1 point for each cusp, which was affected by calcifications and the total maximum score was 5 when all the cusps were affected. According to the values of calcium score from abdominal aorta laterography, the patients were divided into three groups: group I with calcium score from 0 to 7; group II from 8 to 15; and group III from 16 to 24. Depending on the total vascular calcium score values, patients were divided into three groups: total vascular calcium score from 0 to 10-group I, from 11 to 21-group II and group III - from 22 to 32. Patients were grouped also according to the sum of the total calcium score of the aortic and mitral valves, as follows: I group-from 0 to 1, II - from

2 to 3 and group III-from 4 to 5. Patients were separated into two groups according to their serum alkaline phosphatase (SAF) values: group I-with alkaline phosphatase values in the range of 30 to 120 U/L and II - with values above 120 U/L. According to the morbidity from atrial fibrillation (AF) and/or ischemic heart disease (IHD), the patients were separated into the following groups: I group with a history of AF, group II with presence of IHD without AF and group III-without data for heart disease.

The data from assessed vascular score were compared with SAF values, echocardiographic data, and the presence/absence of AF and/or IHD. The results were processed with IBM SPSS Statistics 19 software; χ^2 and linear regression were used as statistical analysis methods. The results were presented as mean values, $p < 0.05$ was considered statistically significant. Descriptive statistics (mean, percentage, degree, etc.) were used to summarize the data.

Results

Forty-seven patients with CKD (35 males and 12 females) were included in the study. The patients' summarized data; demographic characteristics, laboratory variables, diagnostic findings (mean, standard deviations, percentage) are shown in tables 1 through 5.

Table 1. Summarized patients' data. (abbrev. eGFR – estimated glomerular filtration rate)

Patients mean age in years	Average eGFR (5 - 44 ml/min/1.73 m ²)	CKD average duration in months before trial enrollment	Arterial hypertension average duration in months before trial enrollment
62.7±16	21.91±8.9	75.62±62.4	216.7±130.8

Table 2. Mean summarized patients' data. (abbrev. SAF - serum alkaline phosphatase)

SAF values (43 - 209 U/L)	Mean ejection fraction values (38-75%)	serum calcium (1.68 – 2.64 mmol/l)	serum phosphorus (0.63 – 2.40 mmol/l)	calcium phosphorus product (1.73 – 5.6 mmol/l ²)
97.6±33.8	61.8±8.1%	2.25±0.19	1.32±0.37	2.97±0.82

Table 3. Estimated average calcium score. (abbrev. AoV-aortic valve; MV-mitral valve)

Mean abdominal aorta score	Average score (hands, iliac and femoral arteries X-ray)	Mean total vascular score	MV calcifications average score	AoV calcifications average score	AoV and MV total average score
6.47±7.77	1.57±2.49	8.77±8.8	0.68±0.83	0.72±0.94	1.43±1.55

Table 4. Patient distribution according calcium score. (abbrev. AoV - aortic valve; MV - mitral valve)

Groups according abdominal aorta calcium score			Groups according total vascular calcium score			Groups according total AoV and MV calcium score		
I Group	II group	III group	I group	II group	III Group	I group	II group	III group
30 patients (60,8%)	9 patients (19,10)	8 patients (17%)	32 patients (68,1%)	10 patients (21,3)	5 patients (10,6)	31 patients (66%)	8 patients (17%)	8 patients (17%)

Tabl.5. Patient distribution according SAF (serum alkaline phosphatase) values and heart morbidity. (abbrev. AF-atrial fibrillation; IHD-ischemic heart disease)

Patients groups according SAF values		Patients distribution according the morbidity of AF, IHD and no history for heart disease		
I group	II group	AF	IHD	No history for heart disease
37 patients (78,7%)	10 patients (21,3%)	11 patients (23,4%)	15 patients (31,9%)	21 patients (44,7%)

There was a clinically significant relationship between the grades of the abdominal aorta calcium score and eGFR (ml/min/1.73 m²) according MDRD formula ($p < 0.05$). The patients with higher grades abdominal aorta calcium score had lower eGFR (Figure 1A). The same result was obtained when comparing the total calcium score grades and eGFR stage distribution ($p < 0.05$) (Figure 1B).

When comparing the grades of the abdominal aorta calcium score and serum alkaline phosphatase, it was found that there was a moderate correlation in all three patient groups (Cramer's coefficient 0.38), $p < 0.05$. Patients with serum alkaline phosphatase values equal to or greater than 120 U/L had a higher calcium score (Figure 2).

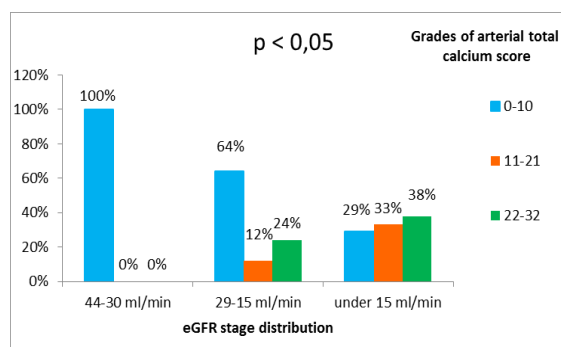
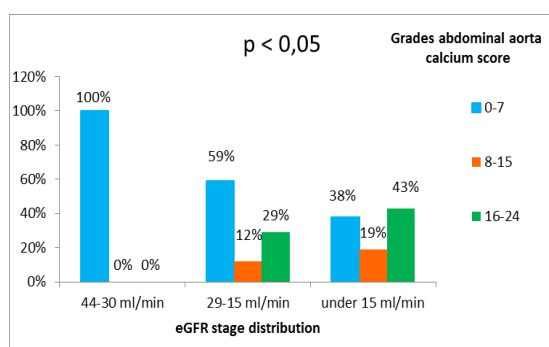


Fig. 1. Correlation between abdominal aorta calcium score grades and eGFR (A). The connection between the grades of the total vascular calcium score and eGFR stages (B).

Clinically significant was the correlation between the grades of arterial total calcium score and the rates of aortic and mitral heart valves total calcium score ($p < 0.05$), with a moderate relationship (Cramer's coefficient 0.40). With increase of the arterial total calcium score, the total valve calcium burden also increased (Figure 3).

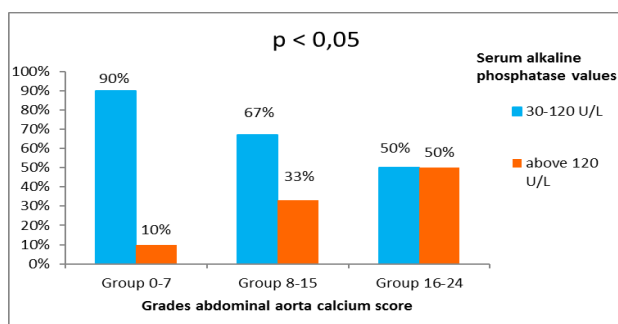


Fig. 2. Relationship between grades of abdominal aorta calcium score and serum alkaline phosphatase

There was a negative correlation [coefficient B (-0.34)] between the abdominal aorta calcium score and the heart ejection fraction (EF) ($p < 0.05$). R Square was 0.105, which means that 10.5% of the EF variations were associated with abdominal aorta wall calcifications (Figure 4).

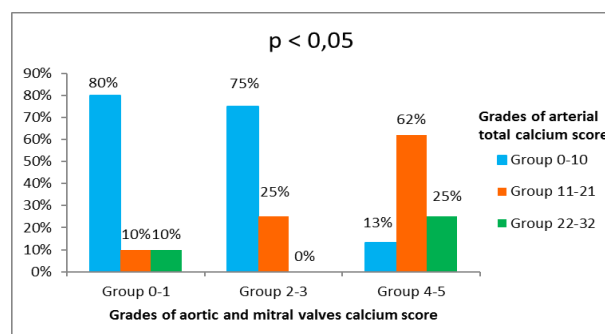


Fig. 3. Relationship between the grades of arterial total calcium score and the total aortic and mitral valve scores rates

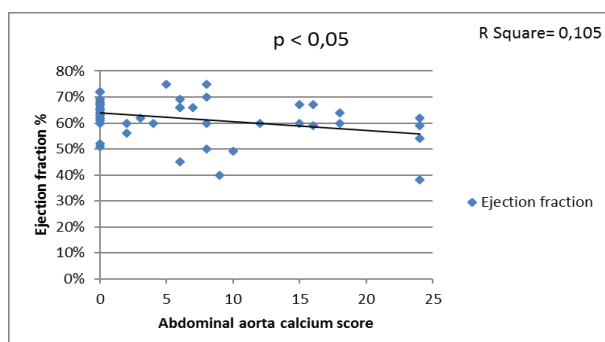


Fig. 4. Linear regression analysis between abdominal aorta calcium score and ejection fraction

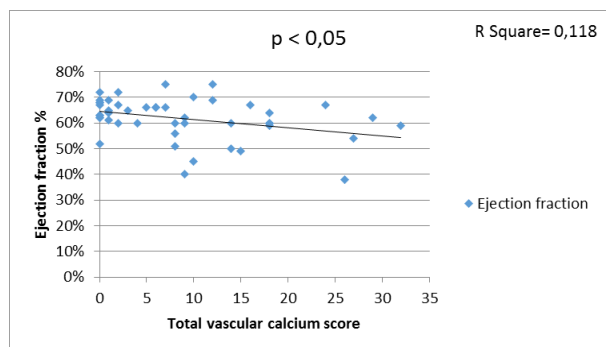


Fig. 5. Correlation between total vascular calcium score and ejection fraction

The relation [coefficient B (-0.34)] between arterial calcium score and heart EF was negative ($p < 0.05$). R Square was 0.118, with 11.8% of the EF variations due to calcifications in the arterial vascular wall (Figure 5). The obtained results showed that 0.3 points increase of the artery calcium burden lead to 1% decrease in the EF, e.g. expressed in whole numbers-increase with 3 points in total vascular score lead to 10% decrease in the EF. There was a moderate correlation (Cramer's coefficient was 0.37) between the grades of abdominal aorta calcium load and cardiac involvement from AF and/or IHD ($p < 0.05$). The data showed that as the calcium score increased, the morbidity of patients with IHD and/or

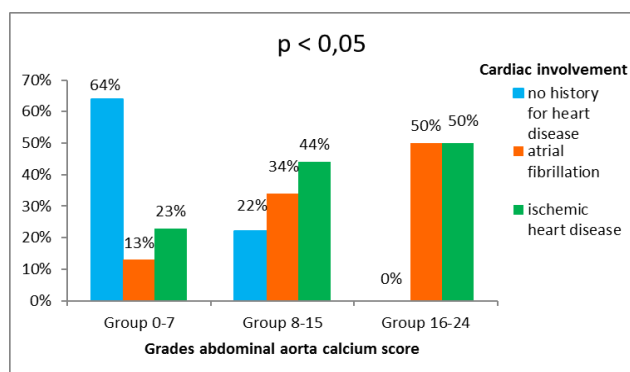


Fig. 6. Correlation between the grades of the abdominal aorta calcium load and the patients' morbidity of the ischemic heart disease and/or atrial fibrillation

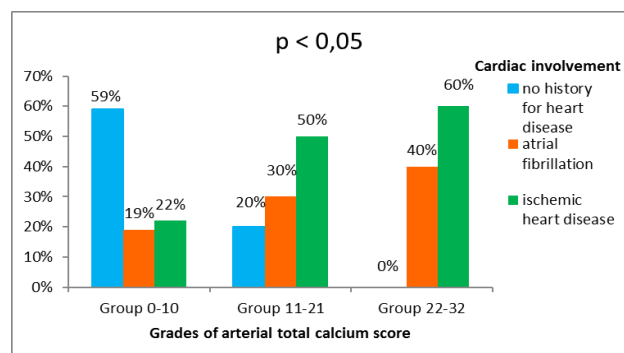


Fig. 7. Relationship between the total arterial calcium score grades and the incidence rate of ischemic heart disease and/or atrial fibrillation

AF increased also (Figure 6). A clinically significant relationship was also found ($p < 0.05$) between the total calcium score from X-ray of the arteries and cardiac involvement from IHD and / or AF (Figure 7).

Discussion

In our study, vascular calcifications were found in the walls of arterial vessels in all of the observed patients. The patients with lower glomerular filtration rate who had advanced chronic kidney disease had a higher abdominal aortic calcium score and higher total vascular calcium grade (Figure 1A and 1B). AC were found more frequently in patients with upper and higher serum alkaline phosphatase values greater than 120 U/L (Figure 2). The data support those of Ronney Shantouf *et al.* who found that serum alkaline phosphatase values above 120 U/L were a strong predictor of greater coronary artery calcification in dialysis patients [30]. Srinivasan Beddhu *et al.* showed that, independently from serum calcium and phosphorus, higher levels of serum alkaline phosphatase were associated with increased mortality in the CKD population [31]. L. Deborah *et al.* support this data in hemodialysis patients [32]. Another study by Blayney J, *et al.* found a strong relationship between increased SAF and higher risk of hospitalization and mortality, independently of serum phosphorus, calcium and parathyroid hormone levels [33]. From the obtained results, it can be concluded that higher SAF values in CKD patients are a predictor of arterial walls calcification, which may be associated with a higher risk of cardiovascular diseases.

We found that a higher vascular calcium score was associated with a higher cardio-valvular calcium burden, which corresponds to a lower EF of the heart and an increased morbidity of AF and/or IHD (Figure 3, 4, 5, 6 and 7). Phan O *et al.*, Schwaiger JP *et al.*, Walsh CR *et al.*, Okuno S *et al.* found that vascular calcifications increase cardiovascular risk, survival and mortality, and were associated with myocardial infarction and congestive heart failure in CKD patients [15,16,18-21]. These data support the studies conducted by Blacher J *et al.*, Otto CM *et al.*, Wang AY *et al.*, Wilson PW *et al.*, Pohle K *et al.*, according who the severity of vascular and valve calcifications were one of the determining factors for survival and mortality, as well as the number and severity of CKD patients' hospitalizations [13,14,22-25]. Qunibi WY *et al.*, London GM *et al.*, Raggi P *et al.*, Panuccio V *et al.* showed that calcium deposits on different cardiovascular structures may be associated with increased morbidity and mortality. The heart valves calcifications may lead to the development of heart failure, coronary ischemia, arrhythmias, valve stenosis, increased risk of infectious endocarditis and thromboembolic events. The valves calcifications were an independent predictor, which can

induce an increased mortality, predominantly from cardiovascular diseases [6,26-29].

Conclusion

It can be summarized, that the echocardiography and X-ray allow accurate and quantitative determination of vascular and valve calcifications. Abdominal aortic laterography is proposed by a group of experts [34] as a reliable method of verifying vascular calcifications and subsequent complications and has good correlation with calcium score determined by computed tomography [35]. Other methods such as electron beam or spiral computed tomography are usually inaccessible to routine practice [17]. The safety and low cost of ultrasound imaging make the echocardiography easily achievable in daily practice and is the gold standard for heart valves morphological and function evaluation: it is not invasive, does not expose the patient to radiation, and is a moderately expensive method. Valve calcification echocardiography and/or abdominal aorta laterography with subsequent calcium burden assessment could be a surrogate marker. Each of these indicators, together with CKD stage determination by eGFR according to MDRD formula could help in clinical practice, easier to determine and predict the cardiovascular morbidity and mortality risk.

Conflict of interest statement: None declared

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*Original article***New Approach in Creating an Arteriovenous Fistula: First Year of Experience Implementing the New Method in R. N. Macedonia**

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Abstract

Introduction. Arteriovenous fistula (AVF) for Hemodialysis (HD) is a surgical anastomosis between the native blood vessels, and it has become a symbol for superior vascular access (VA) for patients on HD. The aim of our study was to compare the successful creation and adequate maturation of AVF using vessel loops instead of arterial bulldogs (small metallic vascular clamp) during surgical intervention.

Methods. The study was retrospective, analyzing the outcome of AVF in patients with Chronic Kidney Disease (CKD) stage 4/5 who underwent AVF creation after hospitalization at our clinic. In 2016 we used arterial bulldogs, and in 2018 we used vessel loops during surgical intervention. Three different types of AVF were created: the distal (radial-cephalic), middle-forearm (radial-cephalic) and proximal (brachial-cephalic). The successful creation and adequate maturation of AVF was defined as successful cannulation of AVF for efficient HD treatment, and AVF blood flow of ≥ 600 ml/min measured by Doppler ultrasound (DUS).

Results. We analyzed 341 created fistulas during the period from January to December 2016, and 367 fistulas created during the period from January to December 2018 in patients with CKD stage 4/5. In 2016 the average age was 62.2 ± 11.2 years, mean HD vintage was 12.8 ± 23.2 months, and 57.5% (196/341) were males. The successful creation and adequate maturation was achieved in 58.65% (200/341), but in preventive fistulas successful creation and adequate maturation was 69.8%. In 2018 the average age was 61.4 ± 14.2 years, mean HD vintage was 14 months, and 64.85% (238/367) were males. The successful creation and adequate maturation were achieved in 74.11% (272/367), but in preventive fistulas successful creation and adequate maturation was 82.69%. In both years the distal location of AVF was predominate site with higher percentage of successful creation and adequate maturation. The smallest percentage of successful creation and adequate maturation of AVF was found in

middle-forearm location, and it correlates with deep location of radial artery that lead to kinking of the anastomosis.

Conclusion. The surgical technique during the AVF creation plays important role. Our analysis showed that creation of AVF was better in 2018 when we used vessel loops, in comparison to 2016 when we used arterial bulldogs. The atraumatic needles, microsurgical instruments and vascular loops decreased mechanical stress in the blood vessels. Use of the vessel loops provides lower mechanical trauma of blood vessels than metallic vascular clamp, with adequate bleeding control.

Keywords: Arteriovenous fistula, surgical anastomosis, vessel loops

Introduction

Arteriovenous fistula (AVF) for Hemodialysis (HD) is a surgical anastomosis between native blood vessels, and it has become a symbol for superior vascular access (VA) for patients on HD. For the patients, VA is the “umbilical cord” keeping them alive, but it can be a constant source of stressful experience as well [1]. A successful creation and an adequate maturation, can provide efficient HD treatment and long-term patients’ survival. Compared with prosthetic graft and central venous catheters, AVF is associated with lower incidence of complications and longer survival [2,3]. The Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines recommended that patients with glomerular filtration rate (GFR) of less than 30 ml/min/1.73 m² or Chronic Kidney Disease (CKD) stage 4/5 should be educated on all modalities of renal replacement therapy (RRT): hemodialysis (HD), peritoneal dialysis (PD) and transplantation (Tx), and an AVF should be placed at least 6 months prior the anticipated start of HD treatment [3].

The first AVF was created by Brescia, Cimino and Appel in 1966 [4]. After an AVF creation, vascular

remodeling occurs with hemodynamic and morphological changes in the blood vessels, that leads to increased diameter and wall thickening and thus, allowing an adequate blood flow for efficient HD [5]. The hemodynamic and morphological changes are the biggest in the first 24 hours after creation. However, AVF still presents a significant medical challenge for the specialists involved in its creation and maintenance. The continuing contemporary technological development in medicine provides better quality of life for patients on HD, but also leads to increased number of newly created VA for HD worldwide. It is essential for all patients to be individually evaluated before the VA creation, in order to secure the most appropriate VA for HD.

In N. Macedonia arteriovenous fistulas are created by a nephrologist since 1976. The Department of Vascular Access, at the University Clinic of Nephrology in Skopje, is the leading institution in the field of VA creation for patients on HD in N. Macedonia which created almost 90% of all VA in the country [6]. The process of AVF creation is surgical intervention, that is performed under local anesthesia (2% Lidocaine) with construction of a termino-lateral anastomosis (end of vein to side of artery) with continuous polypropylene sutures (6-0 Prolene) using microsurgical instruments and 3.5 times magnifying glasses [7].

The aim of our study was to compare the successful AVF creation after using vessel loops instead of arterial bulldogs (small metallic vascular clamp) during the surgical intervention.

Material and methods

The study was retrospective which analyzed the outcome of AVF in patients with CKD stage 4/5 who underwent AVF creation in 2016 and in 2018, hospitalized in University Clinic of Nephrology, Skopje, N. Macedonia. Three different types of AVFs were created: the distal (radial-cephalic), middle-forearm (radial-cephalic) and proximal (brachial-cephalic). Primary thrombosis of AVF was defined as an immediate failure of fistula within 24 hours of creation and primary failure was defined as thrombosis of fistula within 3months. The successful creation and adequate maturation of AVF was defined as successful cannulation of AVF for efficient HD treatment, and AVF blood flow of > 600 ml/min measured by Doppler ultrasound (DUS).

Protocol of Arteriovenous fistula creation

The process of creation of AVF began after complete physical and mental preparation of the patients in aseptic operating room. The surgical intervention was performed under the local anesthesia (2% Lidocaine). We used combination of 10 ml 2% Lidocaine and 10 ml NaCl 0.9% in previously selected location on forearm by DUS. A vein diameter >2.0 mm and an artery

diameter >1.6 mm was considered adequate. We used DUS (Mindray® DC-T6 2010) which was equipped with a linear probe with a minimum frequency of 7 MHz for B-mode. Calculation of the AVF blood flow by DUS used the following formula: vascular area x mean velocity x 60, and blood flow was expressed in milliliters per minute (ml/min).

First, a longitudinal 4-5 cm skin incision was made along the course of artery and vein (distal, middle and proximal side of forearm). From the selected cephalic vein, a segment of 3-5 cm in length was isolated from the surrounding tissue (Figure 1).



Fig.1. Location of blood vessels



Fig. 2. Gentle tension by vessel loops

The separation was done with the help of vessel loops that were placed around the vein with Kelly tweezers. Usage of vessel loops allowed good vascular control, gentler clamping and it eased locating venous branches that were usually removed to improve the mobility of the vein. Then, the focus was on the artery that was exposed with her concomitant veins below fascial fiber. The artery was carefully mobilized with vessel loops in the same manner as the vein. The gentle lift of the artery with vessel loops provided tension in the tissues, thus facilitating the dissection and avoiding injuries (Figure 2). Gentle handling was essential in mobilization of both, the artery and vein. We used the small angle-microsurgery instruments and vessel loops to elevate the vessels that provided adequate vascular control and retracted the artery and the vein without trauma. First, the cephalic vein was cut on the most distal part and the end of the vein was punctured with a hoarsely needle, and distended with 20 ml heparinized NaCl 0.9% solution, 3 times with proximal compression (Figure 3). The adequate distension of the vein was confirmed with minimal resistance to infusion fluid.



Fig. 3. Distention of the vein

The radial artery was usually exposed at the medial side of the incision and was retracted with vascular loops from both sides. The arterial bulldogs (small metallic vascular clamps) were used in brachial artery, when huge calcification was found inside the arteries and in arteries with diameter size bigger of 40 mm. After ensuring good control of the artery with vascular loops, a small incision on the artery was made (Figure 4). The length was 10 mm for radial and 5 mm for brachial artery. In attempt to prevent muscle spasm, mechanic dilatation of radial artery was performed with

passing a blunt-ended probe or artery dilatator, but only in radial artery, and not in brachial artery.

Adequate length of both vessels is necessary for the anastomosis to be made without tension. Also, the angulation of anastomosis is important to avoid later kinking. Properly constructed end-to-side anastomosis

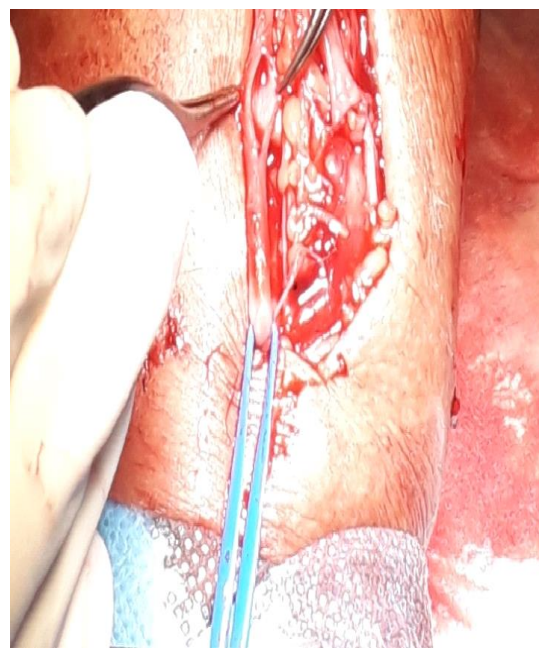


Fig. 4. Lateral incision of artery



Fig. 5. Termino-lateral (end-to-side) anastomosis

resulted with the highest fistula blood flow and minimal distal venous hypertension. The termino-lateral (end of vein to artery side) was the preferred anastomosis (Figure 5), using continuing polypropylene su-

ture (6-0 Prolene) with the aid of 3.5 times magnifying loop. At this stage, we used the heparinized solution for washing the lumen of the artery and vein for removal of clots. A palpable thrill was used as an indicator for successful AVF creation. Palpable pulsation and the absence of the thrill or bruit indicate outflow obstruction and may signal impending thrombosis. After the operation, patients start with exercises with a ball on the same arm where the AVF is. The skin incision was covered with light gauze and dressing.

After the AVF creation, we performed monitoring of AVF maturation with DUS according the current guideline, that define a mature access as one that has a blood flow of at least 600 mL/min, diameter of fistula vein 6 mm and less than 6 mm below the skin surface "Rule 6" [3].

Results

We analyzed 341 created fistulas in 2016 and 367 created fistulas in 2018 in patients with CKD stage 4/5 in one nephrology facility. We compared the successful creation and adequate maturation of AVF between 2016, when we used arterial bulldogs and 2018 when we used the vessel loops.

Three hundred and forty-one fistulas were created in patients with CKD stage 4/5 during the period from January to December 2016. Mean age was 62.2 ± 11.2 years and mean HD vintage was 12.8 ± 23.2 months. From those, 57.5% (196/341) were males, and 42.5% (145/341) were females. The primary cause for CKD in all patients was as it follows: Diabetes mellitus (DM) $n=100$ or 29.33%; Obstructive Nephropathy (ON) $n=57$ or 16.72%; Hypertension (HTA) $n=53$ or 15.54%; Glomerulopathy (GP) $n=26$ or 7.62%; Autosomal dominant polycystic kidney disease (ADPKD) $n=23$ or 6.74%; and Other $n=82$ or 24.05% (Table 1). The distal AVF

was dominant location in 50.4% ($n=172$) of created fistulas, followed by middle-forearm location in 34% ($n=116$) and proximal in 15.6% ($n=53$) of created fistulas. The successful creation and adequate maturation were achieved in 58.65% (200/341). The primary failure within 3 months of creation occurred in 41.35% (141/341), in which early thrombosis or thrombosis with 24 hours after creation occurred in 30.20% (103/341) (Table 2). The successful creation and adequate maturation of AVF per location was: 68.60% (118/172) in distal AVF (radio-cephalic); 43.10% (50/116) in middle-forearm AVF (radio-cephalic) and 60.38% (32/53) in proximal AVF (brachio-basilic) (Table 3). Preventive fistulas were 15.8% ($n=54$) and successful creation and adequate maturation was presented in 69.8% of the total number of created fistulas.

Table 1. Demographics characteristics and distribution of primary renal disease of created fistulae

	2016	2018
Number	341	367
Males	196(57.5%)	238(64.85%)
Females	145(42.5%)	129(35.15%)
Mean age (years)	62.2 ± 11.2	61.4 ± 14.2
HD vintage(months)	12.8 ± 23.2	14
<i>Etiology of renal disease</i>		
Diabetes Mellitus (DM)	100(29.33%)	102(27.80%)
Other	82(24.05%)	104(28.34%)
Obstructive Nephropathy (ON)	57(16.72%)	39(10.63%)
High blood pressure or Hypertension (HTA)	53(15.54%)	71(19.36%)
Glomerulopathy (GP)	26(7.62%)	34(9.27%)
Autosomal dominant polycystic kidney disease (ADPKD)	23(6.74%)	17(4.6%)

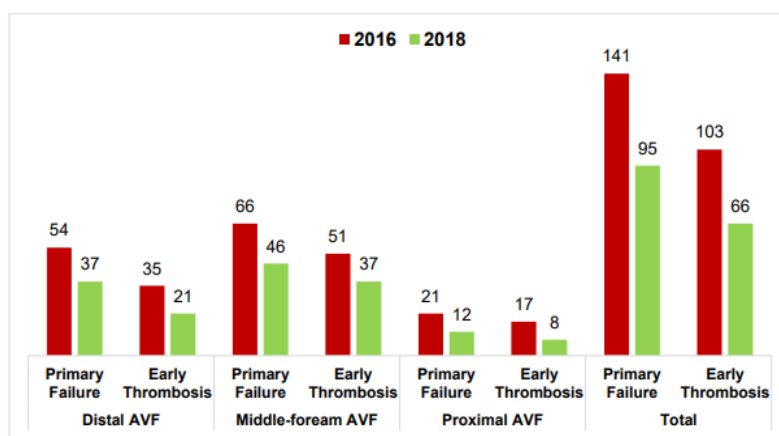


Fig. 6. Early Thrombosis from Primary Failure of AVF by location

In comparison, during the period from January till December 2018, 367 fistulas were created. Mean age was 61.4 ± 14.2 years and mean HD vintage was 14 months, and 64.85% (238/367) were males. Also we

analyzed the primary cause for chronic kidney disease in these patients: Diabetes mellitus (DM) $n=102$ or 27.80%; Obstructive Nephropathy (ON) $n=39$ or 10.63%; Hypertension (HTA) $n=71$ or 19.36%; Glomerulopathy

(GP) $n=34$ or 9.27%; Autosomal dominant polycystic kidney disease (ADPKD) $n=17$ or 4.6%; and Other $n=104$ or 28.34% (Table 1). The distal AVF was dominant location in 45.78 % ($n=168$) of created fistulas same as 2016, followed by middle-forearm location in 37.06% ($n=136$) and proximal in 17.16% ($n=63$) of created fistulas. The successful creation and adequate maturation were achieved in 74.11% (272/367). The primary failure within 3 months of creation occurred in 25.89% (95/367), in which early thrombosis or thrombosis within 24 hours

of creation, occurred in 17.98 % (66/367) (Table 2). Successful creation and adequate maturation of AVF per location was: 77.98% (131/168) in distal AVF (radio-cephalic), followed with 66.17% (90/136) in middle-forearm (radio-cephalic) and 80.95% (51/63) in proximal AVF (brachio-basilic) (Table 3). Preventive fistulas were 14.17% ($n=52$) and successful creation and adequate maturation was presented in 82.69% of created fistulas.

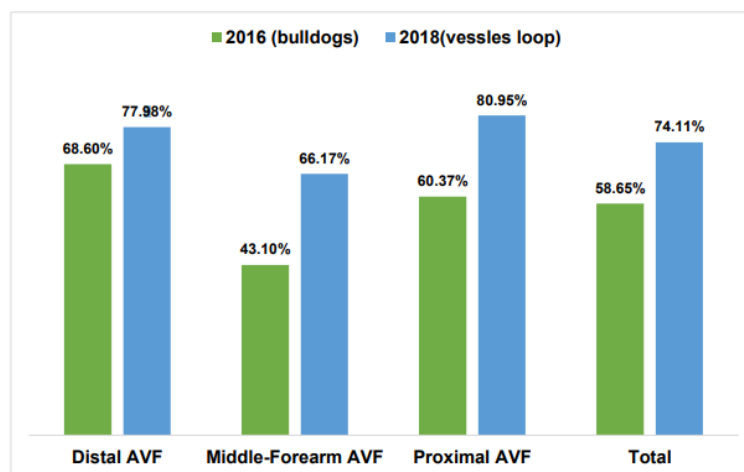


Fig 7. Comparison of the creation and maturation of AVF

Discussion

The AVF remains the preferable choice for VA in patients on HD, being associated with lower complication and longer patency rate, in comparison to the tunneled central venous catheters and synthetic grafts. In addition, VA failure is a common and serious complication leading to increased temporary catheter use, access creation at multiple sites, and after many years of multiple access failures, catastrophic inability to undergo HD in some cases. The goal of the specialist involved in creation of AVF is to obtain blood flow volume ≥ 600 mL/min that allows adequate HD treatment. Careful planning of VA is of crucial importance for patients on RRT. Physical examination and pre-operative vascular mapping with DUS determine the forearm location of AVF creation [8]. Also, the surgical technique during the AVF construction plays an important role. Appropriate length of the blood vessels and reduced angulation between them, avoids kinking of the vessels [9]. The atraumatic needles, microsurgical instruments and vascular loops decrease mechanical stress in the blood vessels. Regarding the above, creation of AVF is still a complex procedure that requires multidisciplinary approach. Building and nurturing a team of dedicated VA specialists may be what maximize success. Surgeons who had created at least 25 arteriovenous fistulas during training had significantly lower rates of AVF failure than those placed by sur-

geons who had created fewer than 25 with a relative risk of 0.66 [10]. It allows team members to gain experience in the various techniques available and to monitor success as well as complication rate at a local level. Laskar *et al.* compared continuous versus interrupted suture of AVF in 40 participants. Access survival at 2 years, was found to be similar in both groups [11]. One of the criticisms of the interrupted suturing technique is that it is more time-consuming [12]. Lykoudis *et al.* in their study, were using combined technique in suturing-interrupted sutures around the heel of the anastomosis and then continuous sutures for the rest [13]. In our study, we used the continuous polypropylene sutures (6-0 Prolene) using microsurgical instruments and 3.5 times magnifying glasses for anastomosis of AVF creation. Three randomized control trials (RCTs) that compared clips versus sutures in performing end-to-side anastomosis for AVF creation, but all three found uncertain effects on primary patency or AVF maturation [14-16]. Also, no study addressed end-of vein to end-of artery anastomosis or other newer techniques that are less often performed [1]. We used the end-of vein to side-of artery (terminolateral anastomosis) for AVF creation.

In our analyses from 2018 we used the vessel loops, the successful creation and adequate maturation of AVF was 74.11% (272/367) and the primary failure within 3 months was 25.89% (95/367). Going backwards in 2016 on the other hand, when we used arterial bulldogs,

the successful creation and adequate maturation of AVF was 58.65% (200/341), and the primary failure within 3 months was 41.35% (141/341). When the two periods were compared, the creation of AVF seemed better in 2018 when we used vessel loops in comparison to 2016 when we used arterial bulldogs, and the distal location of AVF was dominate with highest percentage of successful creation and adequate maturation. The smallest percentage of successful creation and adequate maturation of AVF was found in middle-forearm location, and it correlates with deep location of radial artery that lead to kinking of the anastomosis. We have been encouraged to present our first experience in using this new method in AVF creation. In general, it could have been a small step for the mankind but for us it was a novelty, and further analysis are certainly required in order to know the benefit of it. Reduction of the mechanical trauma during the AVF creation increases the blood flow in the artery leading to an increased in wall shear stress and tension. The blood flow and wall shear stress increase substantially in the vein. This increased flow results in a venous luminal diameter increase, a process necessary for cannulation. The wall shear stress gradually returns back to normal within 12 weeks [17]. Also, the vessel loops generate a lower pressure on smooth endothelial cell lining the tunica intima, defined as the boundary of the endothelial cell to the elastic lamina in the normal artery. The tunica media in an artery is normally much thicker than a vein with an increased amount of elastin [18]. This group of patients with end stage kidney disease (ESKD), have underlying changes in the vein and artery, including increased arterial and venous calcifications [19]. It seems that it correlates with primary renal disease, where DM occurs as the leading cause for ESKD [20]. When we detected a huge artery area of calcification, we did not use the vessel loops, because the artery wall was very rigid and we cannot provide adequate bleeding control. Also, we did not use the vessel loops in case when the diameter of artery was bigger of 4.0 mm, because we cannot provide adequate bleeding control. In both cases we used the arterial bulldogs for bleeding controls. The last annual registry from 2017 by European Renal Association-European Dialysis and Transplantation Association (ERA-EDTA) showed that DM with 23% is the leading cause for ESKD [21]. In our analyses DM was presented with 29.96% for 2016 and 27.79% for 2018, which makes it one of the leading causes for ESKD. Further analysis is needed for assessment of long term AVF functional capacity, and also early vascular biological events that need to be unraveled. Anastomotic design and strategies and devices to define optimal wall shear stress are in a developing process [22]. Taking that into consideration, the Optiflow (Biocor Systems, Ambler, Pa, USA) is an internal anas-

tomotic connecting device placed inside the artery and vein, with the purpose of improving hemodynamics and standardizing AVF creation. A non-randomized study in 41 patients and 39 matched control patients reported comparable unassisted maturation rates at 14, 42, and 90 days of 76%, 72%, and 68% for the Optiflow group and 67%, 68%, and 76% in the control group, respectively. In this study, there was a trend for earlier maturation in the Optiflow group compared with the control group ($p=0.059$) [23].

There is a high possibility that factors not connected with patients characteristics and surgical skills are important in determining outcomes, and it might possibly explain the apparent contradiction of ESKD practices (Tx, PD, patterns of vascular access use in HD), where some countries excel in one area and score poorly in another.

Conclusion

In summary, the surgical technique during the AVF construction plays important role. Our analysis showed that creation of AVF is superior when we used vessel loops, in comparison when we used arterial bulldogs. The atraumatic needles, microsurgical instruments and vascular loop decreased mechanical stress in the blood vessels. Use of the vessel loops provides lower mechanical trauma of blood vessels than metallic vascular clamp, with adequate bleeding control. Also, we must continue the improvement of AVF functionality, in order to investigate the hemodynamic, morphological and vascular biology of the AVF and in order to develop better clinical parameters.

Conflict of interest statement: None declared

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Original article

Permanent Vascular Access for Hemodialysis in Elderly Patients - Single Center Experience

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Abstract

Introduction. It is estimated that patients aged ≥ 75 represent 20% of the prevalent population on hemodialysis. Even though arterio-venous fistula is widely regarded as the best option for permanent vascular access for hemodialysis, the situation with the elderly population on hemodialysis is a bit more complicated. This study aimed to present the type of permanent vascular access for hemodialysis in these patients created in the last year in our center.

Methods. During the last 12 months in 86 patients aged ≥ 75 , various type of permanent vascular access for hemodialysis was created. Data about the gender, age, comorbidity condition and the type of vascular access was collected from the medical records.

Results. The majority of our patients were male (61%), mean age was 79.2 years. The main permanent vascular access created for hemodialysis was arterio-venous fistula in 49 (57.6%), followed by tunneled cuff catheters in 20 (23.5%) patients, 1 patient had arterio-venous graft, and 16(17.6%) patients had non-tunneled central venous catheter as permanent vascular access for hemodialysis. The main reason for using tunneled and nontunneled catheters were thrombosis, non-maturation of the fistula and poor general condition with low life expectancy, respectively. Forty-five patients (53%) had started hemodialysis in the last year, and in 50 patients (58.8%) the current permanent vascular access was their primary access. The same pattern was seen in the subgroup of patients older than 80 years.

Conclusion. Arterio-venous fistula is the best option for permanent vascular access regardless of the age of the patients. When the conditions are not favorable, such as thrombosis, non-maturation and especially very poor general condition with short life expectancy tunneled cuff catheters and even non-tunneled central venous catheters can be seen as a viable option.

Keywords: vascular access, hemodialysis, elderly patients, single center experience

Introduction

The incident hemodialysis population is ageing, and it is estimated that patients aged ≥ 75 represent 20% of the prevalent population on hemodialysis (HD) [1]. Usually, age above 65 is regarded as an elderly population, but since so many patients are older than 75 years, we decided to use this frame for elderly patients. This population is burdened with a lot of severe comorbidities, so all types of vascular accesses for HD are problematic. Even though arterio-venous fistula (AVF) is widely regarded as a best option for permanent vascular access for hemodialysis, the situation with the elderly population on hemodialysis is a bit more complicated. In the literature several studies are currently dealing with this problem [2-5]. Atherosclerosis and previous vascular damage lead to thrombosis or non-maturation of AVF, which itself can cause or worsen cardiac ischemia and heart failure. Arteriovenous grafts (AVG) require more procedures to maintain patency, on the other hand, fistulas require more procedures for establishing patency, and so overall patency may not differ substantially between the two forms of permanent access [6]. This may be especially important in older adults because of their more limited life expectancy and increased risk of failed fistula maturation [7, 8]. Sometimes, the maturation period for AVF exceeds the lifespan of the patient, so having this fistula presents no benefit for the patient. Some authors question the implementation of the Fistula First Initiative in elderly [8, 9] and some even recommend modifying the guidelines in this population [10]. Vascular access planning in the elderly is different from that in younger patients, and the Fistula First Initiative may not be the preferred approach for older patients because of their reduced life expectancy and conflicting results after surgery [11].

Central venous catheter (CVC), with its known complications, such as increased infections, pose risks of its own, although there are reports that there is a lower

risk for catheter related bloodstream infections in this population, compared to the younger patients, so in some situations, they may represent a suitable option for HD in settings of non-maturing AVF or poorly functioning synthetic grafts [12].

Material and methods

Observational retrospective study was conducted in which we evaluated the prevalent type of permanent vascular access in elderly patients created in our center from December 2018 up to November 2019. Baseline population included all patients who were ≥ 75 years and were presented for creating permanent vascular access. A subgroup of older elderly patients, aged ≥ 80 years was created.

Major comorbidities (diabetes, arterial hypertension, acute myocardial infarction, coronary revascularization, malignancy) were identified using prior patient medical history. After a thorough examination of the general condition of the patient, present comorbid diseases, physical and ultrasound Doppler examination of the arm, the decision was made for the type of permanent vascular access to be created.

Results

In 86 patients aged ≥ 75 years, mean age 79.2 years, 60% of which were male, permanent vascular access was created. Nephroangiosclerosis was found in 34 patients as the leading cause for chronic kidney disease, followed by diabetic nephropathy in 18 patients, multiple myeloma in 6 patients, 3 patients had obstructive nephropathy due to malignancy and 25 patients were evaluated with an unknown diagnosis. Characteristics of the patients studied are presented on Table 1.

The arteriovenous fistula was created in 49 patients (57.6%), 20 patients (23.5%) had inserted tunneled cuff catheters (TCC), 1 patient (1.2%) had AVG, and 16 patients (17.6%) had inserted non-tunneled CVC as permanent vascular access for hemodialysis (Figure 1).

Forty-five patients (53%) were incident patients, 28 (52.2%) of them had AVF as permanent vascular access, 4 (8.8%) TCC and 13 (28.8%) non-tunneled catheters. In 50 patients (58.8%) the current permanent vascular access was their primary access.

The main reason for using tunneled catheters was thrombosis or non-maturation of the AVF. This was seen in ten patients, followed by the general bad condition in four patients. The arterio-venous graft was placed only in one patient who had multiple previous AVF. Non-tunneled CVC as permanent access for hemodialysis was used for patients with the poor general condition, mostly with the terminal malignant disease with short life expectancy, but also with heart failure (Figure 2). The same pattern was seen when we evaluated the pa-

tients older than 80 years. From 36 patients, 63.8% were male, and the most used permanent vascular access was AVF (50%), followed by CVC in 10 (27.7%) and TCC in 8 patients (22.2%) (Figure 3).

Table 1. Characteristics of the study cohort

Baseline characteristics	Patients ≥ 75 years	Patients ≥ 80 years
Age, median	79.2	82.7
Male, n (%)	52 (60)	21 (63.8)
Comorbidities, n (%)		
Nephroangiosclerosis	34 (29.2)	12 (33.3)
Diabetes mellitus	18 (15.5)	7 (19.4)
Multiple myeloma	6 (5.2)	3 (8.3)
Obstructive nephropathy	3 (2.6)	
Unspecified	25 (21.5)	14 (38.9)
Type of permanent vascular access, n (%)		
Overall	86	36
AVF	49 (57.6)	18 (50)
TCC	20 (23.5)	8 (22.2)
CVC	16 (17.6)	10 (27.8)
VG	1 (1.2)	
Main reason for not using AVF, n (%)		
Thrombosis/non-maturation	15 (40.5)	5 (27.8)
Heart failure	7 (18.9)	5 (27.8)
Poor general condition	13 (35.1)	7 (38.9)
Haemostatic disorders	1 (2.7)	1 (5.6)
Arteria brachialis rupture	1 (2.7)	
Patients starting HD		
Overall, n (%)	45 (53%)	16 (44.4)
AVF	28 (62.2)	7 (43.3)
TCC	4 (8.9)	1 (6.2)
CVC	13 (28.9)	8 (50)
Primary permanent vascular access		
Overall, n (%)	50 (58.8)	20 (55.5)
AVF	35 (70)	12 (60)
TCC	5 (10)	2 (10)
CVC	10 (20)	6 (30)

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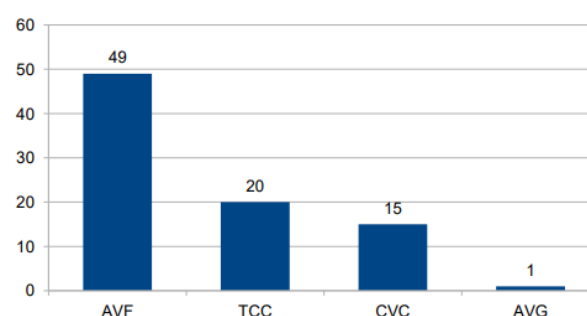


Fig. 1. Type of permanent vascular access in elderly patients

The reasons for using CVC and TCC were the same as described above, poor general condition and thrombosis/non-maturation of the AVF, respectively (Figure 4). Sixteen patients (44.4%) were incident patients, and for 20 patients (55.5%) this was their primary permanent vascular access for hemodialysis.

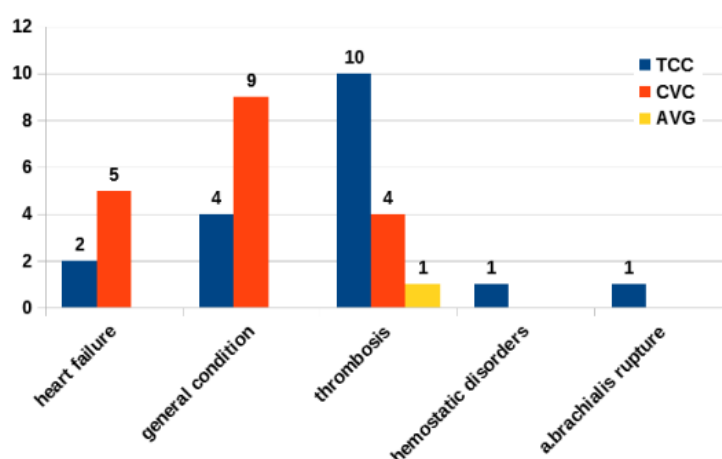


Fig. 2. Main reasons for not having AVF

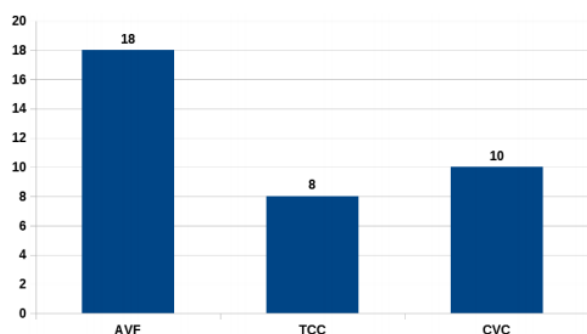


Fig. 3. Permanent vascular access in patients >80 years

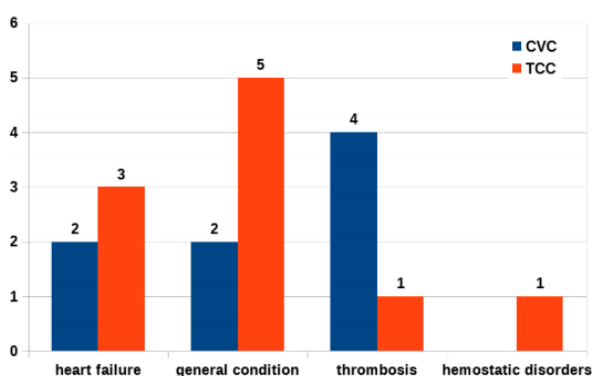


Fig. 4. Main reasons for not having AVF in patients >80 years

Discussion

Every year, the number of elderly patients with chronic kidney disease in need of chronic hemodialysis rises. This study was aimed in presenting the prevalent number of elderly patients with CKD requiring permanent vascular access for hemodialysis in our country. Our center is responsible for care for about 85-90% of all vascular accesses for hemodialysis in R. North Macedonia. From roughly 1500 patients on hemodialysis in R. North Macedonia, 85 elderly patients aged ≥ 75 years (5.6%) needed permanent vascular access last

year. Although various societies' guidelines do not differ between the young and older patients in recommen-

ding AVF as a best choice for permanent vascular access for HD, the situation with the elderly patients is not that simple.

These are patients with many comorbid conditions, poor general condition and poor life expectancy. General poor condition with low life expectancy was the main reason for using TCC and CVC in these patients. There is only one patient with AVG because in our institution we do not insert AV graft. Our vascular surgeons are responsible for vascular grafts, although, generally speaking, the stance in Macedonia regarding vascular grafts is not very favorable. The only AVG inserted in our clinic was done with the help of a vascular surgeon.

The decision which type of access for which the patient relies not just on the blood vessels, but also on the presence of various comorbid conditions and general condition of the patient. The same observations were found in two similar publications. Both Brown *et al.*, [13] and Quinn *et al.*, [14] came to conclusion that difference in mortality between patients with different permanent vascular access is mainly due to conditions that lead the patients to receive certain vascular access rather than complications related to the vascular access itself. Healthier patients received an AVF, and the patients with poorer condition CVC.

Whenever we had the opportunity, we placed AVF in our patients, but when conditions were not favorable, especially in patients with poor life expectancy we chose to insert tunneled or non-tunneled CVC as permanent vascular access for hemodialysis.

Age itself is not a factor for choosing a type of vascular access. In some situations, having AVF can be seen as a marker for patient with a generally good condition and longer survival.

Conclusion

Arterio-venous fistula is the best option for permanent vascular access regardless of the age of the patients. Still, when the conditions are not favorable, such as thrombosis, non-maturation of AVF and especially very poor general condition with short life expectancy tunneled cuff catheters and even non-tunneled CVC can be seen as a viable option.

Conflict of interest statement: None declared

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Case report

Membranous Nephropathy as a Paraneoplastic Syndrome in A Patient with Ovarian Serous Cystadenofibroma - Case Report

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Abstract

The nephrotic syndrome (NS) has been associated with a variety of malignancies in several numbers of literature reports but has been reported in only nine cases associated with ovarian neoplasms so far. Particularly, the ovarian serous cystadenofibroma has not been described so far anywhere in the literature. We describe a case of a 46-year-old woman presented in our Nephrology Department, with biopsy-proven membranous nephropathy, most probably secondary to ovarian serous cystadenofibroma. The surgery (bilateral adnexectomy) and pulse treatment with corticosteroids, led to remission of the NS. To the best of our knowledge, this is the first report of an ovarian serous cystadenofibroma associated with membranous nephropathy, with remission of the NS after tumor removal and pulse steroid treatment.

Keywords: nephrotic syndrome, membranous nephropathy, paraneoplastic syndrome, resolution of nephrotic syndrome

Introduction

The nephrotic syndrome (NS) has been associated with a variety of malignancies in several reports in the literature but has been reported in only nine cases associated with ovarian neoplasms so far. The relationship between glomerulopathy and tumor pathology was first described in 1922 by Galloway [1] in a patient with Hodgkin's disease who developed massive proteinuria. This association was confirmed in 1966 by Lee *et al.* who found 11 patients with cancer in a cohort of 101 patients with a nephritic syndrome [2]. The most common renal pathology in these patients was membranous nephropathy (MN) with deposition of the immune complex [3].

Tumors as an important source of antigens may induce the production of specific antibodies forming immune complexes subsequently deposited in the kidneys. Another

hypothesis is that antigens with a high affinity for basement membrane constituents can get implanted directly into the renal tissue and induce the formation of immune complexes with circulating antibodies [2]. These two mechanisms would explain the role for chemotherapy or surgery in the reversal of the glomerular injury in these settings.

We present a patient with a benign ovarian tumor (serous cystadenofibroma) who suffered from nephrotic syndrome. To the best of our knowledge, nephrotic syndrome has not been described so far in association with serous cystadenofibroma.

Case-report

A 46-year-old woman presented recently in our Nephrology Department with a history of progressive edema of lower extremities and ascites of 7 weeks' duration. From the comorbidities, she had arterial hypertension well controlled with one antihypertensive drug for the last 10 years (Nifedipine 20-40 mg/day), and bronchial asthma since 2013. She was prescribed diuretics at the first consultation with her doctor because of the lower extremities swelling, but with no positive results at all. At her second consultation, the laboratory analysis were in favor of hypoalbuminemia (albumin 17 g/l), normal levels of serum creatinine (creatinine 51 µmol/l), normal markers of inflammation (CRP 1.3, Le 7.2), and elevated levels of tumor markers (CA 125-536.5 U/ml, CA 15-3-45.5 U/ml). The abdominal echotomography showed normal-sized kidneys with bilateral, hyperechoic cortical texture, and also, an ascites presentation. The abdominal CT showed abnormal mass finding on the right ovary. Thereafter, she was hospitalized in the Gynecology and Obstetrics Department, where bilateral adnexectomy was performed as per protocol. Post-operative course was uneventful. During the hospitalization, massive proteinuria was confirmed twice before surgery (30 g/24h; 46 g/dU). The anti-glomerular basement membrane antibodies (anti-GBM) were nega-

tive, and the results from the anti-nuclear antibodies (ANA) and anti-neutrophil cytoplasmic antibodies (ANCA) were in reference values. The chest X-ray showed no abnormality. Serous cystadenofibroma was the histological diagnosis obtained from the Pathology department. The persistence of edema and massive proteinuria after surgery indicated further need for evaluation and treatment in our Nephrology Department. After a careful nephrological workup, a decision for renal biopsy (RB) to determine the precise direction of further treatment, was considered. A diagnosis of NS, most probably MN was suspected. While waiting for the histopathological renal biopsy finding, the patient received pulse treatment of 500 mg of methylprednisolone (for 3 consecutive days, tapering the dose in the following days). She was initially treated with diuretics and supplemented with albumin/plasma due to the persistent hypoproteinemia and hypoalbuminemia. After the corticosteroid pulses, the levels of proteinuria slowly reduced being 11.5g/24h at the hospital discharge. During hospitalization, the histology confirmed features of membranous nephropathy, and the patient continued with oral corticosteroids (Decortin 40 mg/day). Within three weeks of the tumor resection, edema disappeared and serum albumin started to improve. We strongly advised further follow up under treatment of this patient, and anticipate the remission of her nephrotic status in around 6 months after surgery.

Discussion

Different types of glomerular diseases may be associated with tumors. Paraneoplastic NS could be due to MN as presented in our case, minimal change disease, focal segmental glomerulosclerosis, or amyloidosis [4]. Despite the hypothesis of tumor proteins presenting as antigens inducing antibodies to immune complexes formed in the basement membrane, it could not have been certainly established. Additionally, an enhanced immune

reaction triggered by the tumor itself may be required in the development of MN. Another hypothesis is that certain antigens with a high affinity for the basement membrane get implanted in situ and form immune complexes with circulating antibodies. On the other hand, a theory of persistent virus load causing primarily glomerulonephritis and then malignancies, perhaps through common pathogenesis has been also established. Several histological characteristics by immunofluorescence and electron microscopy may help to distinguish between idiopathic and secondary forms of membranous nephropathy [4]. Presence of immunoglobulin G, IgG1 and IgG2 subtypes, is more marked in the kidneys of patients with paraneoplastic membranous nephropathy than in those with idiopathic membranous nephropathy due to activation of both Th1 and Th2 cytokines which may be activated by tumor antigens or other stimulants, resulting in the unique pattern of IgG subtype and increased numbers of inflammatory cells [5]. That glomerulopathy is due to the malignancy and supported by the fact that remission occurs after treatment of the primary etiology (surgical excision or chemotherapy) and relapse of proteinuria occurs after a recurrence of the tumor [5]. Remission of the paraneoplastic nephrotic syndrome was described at various times after the resection of tumors, which is connected with immunological dysregulation in the course of neoplastic disease [6]. Ovarian tumors can be associated with membranous nephropathy, as described in Table 1.

Based on this literature, remission of the NS was seen in 6 cases that experienced successful treatment of an ovarian tumor. In only 3 cases, the association of the paraneoplastic syndrome was with a benign form of a neoplasm [2,9,12], among the other 5 that are connected and explained by the presence of malignancy [2,7,8,10,11] One out of five patients having the malignant type, obtained remission of NS only by excision and steroid treatment [1]. On the other hand, two out of three patients having the benign form reached to remission

Table 1. Evidence of ovarian tumors associated with MN

Authors	Age	Kidney histology	Ovarian tumour type	Surgery Steroid	Treatment Chemotherapy	Response of NS
Lee <i>et al.</i> [2]	65	MN	Adenocarcinoma	No	No	No
Lee <i>et al.</i> [2]	28	MN	Benign teratoma	Yes	No	Yes
Torres <i>et al.</i> [7]	N/A	MN	Adenocarcinoma	Yes	N/A	Yes
Hoyt <i>et al.</i> [8]	65	MN	Papillary serous carcinoma	Yes	Yes	N/A
Jeroydi <i>et al.</i> [9]	36	MN	Benign mature cyst teratoma	Yes	No	Yes
Forgy <i>et al.</i> [10]	68	MN	Serous adenocarcinoma	Yes	Yes	No
Ata <i>et al.</i> [11]	65	MN	Clear cell carcinoma	Yes	Yes	Yes
Kilis-Pstrusinka <i>et al.</i> [12]	16	MN	Benign mature teratoma	Yes	No	Yes

by extracting the tumor together with corticosteroids [9,12], without the need of chemotherapy as well. In contrast to these findings, remission of the NS was not achieved in two patients with ovarian tumor (adenocar-

cinoma and benign teratoma) even though one of them was treated with prednisone and tumor excision as per recommendations [2]. In our patient, the surgical resection of the ovarian tumor and corticosteroid treat-

ment [13] resulted in brisk remission of the nephrotic syndrome although we would underline that methylprednisolone alone is not supported by evidence, or by KDIGO guidelines with good effect in MN. The possibility of a coincident occurrence of these conditions cannot be completely ruled out. However, considering the patient's age, the lack of other causes of nephrotic syndrome, the consecutive association between tumor diagnosis and the onset of symptoms as well as the remission of the NS following treatment of the ovarian mass seems to be a reasonable consideration.

Conclusion

Association of the NS with benign ovarian tumors is very rare, with currently available reports from three cases. Ovarian serous cystadenofibroma precisely has not been described so far anywhere in the literature. Paraneoplastic NS has membranous glomerulonephritis as its most common pathology. Possible hypotheses include immune complex reactions induced by either tumor antigen or viral infection. In our case, remission of the NS occurred after excision of the tumor along with the corticosteroid treatment, support to a certain extent the etiological role of the tumor. Finally, the treatment of this kind of patients is a great challenge requiring a multidisciplinary approach.

Conflict of interest statement: None declared

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Case report

Successful Kidney Transplantation from a Deceased Donor with Bacterial Meningitis Caused by *Streptococcus Pneumoniae*

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Abstract

Kidney transplantation is considered the best treatment for patients with end-stage renal disease because it provides longer life expectancy and better quality of life when compared to dialysis. A shortage of organs for transplantation has led to the increase of waiting lists and, consequently, to the expansion of criteria for suitable organ donors. Therefore, bacterial meningitis in a potential donor is not considered an absolute contraindication for kidney transplantation. We present the patient who received the kidney from a deceased donor who died of bacterial meningitis caused by *Streptococcus pneumoniae*.

Key words: kidney transplantation, donor, meningitis, *Streptococcus pneumoniae*

Introduction

Kidney transplantation is the best treatment for patients with end-stage renal disease because it provides longer life expectancy and better quality of life when compared to dialysis. A limited number of organs for transplantation has led to the increase of waiting lists and, consequently, to the expansion of criteria for suitable organ donors. Nowadays, deceased donors with proven active infections may be considered for organ donation [1]. We present the patient who received the kidney from a deceased donor who died of bacterial meningitis caused by *Streptococcus pneumoniae*.

Case report

A 42-year-old male developed an end-stage renal disease caused by Alport syndrome. At the age of 2 years, he had microscopic hematuria, but no further diagnostic examination has been done during the next 19 years. In 1999, erythrocyturia with 80% dysmorphic erythrocytes was found, without proteinuria. Biopsy

revealed Alport syndrome. He has undergone haemodialysis since June 2019.

On 25th January 2020, our centre received an offer from the Eurotransplant. The brain-dead donor was 35-year-old, male, who died of bacterial meningitis caused by *Streptococcus pneumoniae*. Cardiopulmonary reanimation was performed at emergency department due to respiratory arrest. His medical history was uneventful. Allergy to penicillin demanded use of vancomycin 1x1g with meropenem 3x2g for 10 days. Laboratory parameters of kidney function were within the normal range, with CRP 81.9 mg/L. Abdominal ultrasound showed normal size and shape of both kidneys. He spent 11 days in the Intensive care unit and had a hypotension period of 30 minutes. Heart, liver and kidneys were offered for transplantation.

The recipient was informed that transplantation of a kidney from the infected donor increases the risk for the infection during the post-transplantation period because of the immunosuppressive therapy. Additionally, he was warned that more prolonged antibiotic prophylaxis would be needed. As the patient accepted the risk, the informed consent was obtained. He was admitted in hospital as a potential recipient of a cadaveric kidney. Miss match was 2, 0, 1. The crossmatch testing was negative.

As there were no contraindications from the recipient, kidney transplantation was done with the double-J (JJ) stenting of the ureter. The patient received antibiotic prophylaxis for 14 days, including amoxicillin/clavulanic acid 1 g twice daily starting before transplantation and continuing until the 10th post-transplant day, followed by cefuroxime 250 mg twice daily until the 14th post-transplant day. Immunosuppressive treatment included the induction with basiliximab, tacrolimus, mycophenolate mofetil and prednisone. An adequate graft function has been shown early post-transplant, with satisfactory diuresis on the 3rd post-transplant day (2000 ml/day) and serum creatinine of 200 µmol/L on the day of dismissal at the 9th post-transplant day. Two months after transplantation serum creatinine decreased

to 99 $\mu\text{mol/L}$. There were no signs of infection during the post-transplant period.

Discussion

Kidney transplantation from infected donors carries the risk of the transmission of the infection to the recipient, mainly because of the immunosuppressive therapy. Deceased donors with meningitis and encephalitis have been considered with special attention, particularly since two renal transplant recipients who died of encephalitis of unknown cause transmitted from the same donor have been reported [2,3]. However, there are several reports of successful kidney transplantations with organs from donors who died of bacterial meningitis caused by *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Neisseria meningitidis* and *Escherichia coli* [4-9]. In these cases, donors have been treated with antibiotics directed to the cultured bacteria for 24-48 hours before transplantation, and the recipient has also received antibiotic prophylaxis according to antibiogram for 7-14 days post-transplant. Therefore, most guidelines stress the importance of appropriate prophylactic anti-microbial therapy and warn that the risk of infection transmission is higher when the causative agent of the donor's infection is not known [2].

Our case demonstrates that kidneys from a deceased donor who died of meningitis caused by *Streptococcus pneumoniae* may be suitable for transplantation if the donor has received antimicrobial therapy according to antibiogram and if the recipient follows adequate antibiotic prophylaxis for a sufficient period.

Conclusion

Bacterial meningitis in a potential donor is not considered an absolute contraindication for kidney transplantation. Adequate antimicrobial therapy administered to

donor and adequate recipient prophylaxis minimizes the risk of infection transmission. Therefore, kidneys from donors with bacterial meningitis should be considered for transplantation and the clinician's decision is based on the evaluation of the ratio between potential benefit and the potential risk for each patient.

Conflict of interest statement: None declared

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