Assessment of chronic kidney disease based on necroptic microscopical examination in violent death cases

Observations on five-year casuistry of the Institute of Legal Medicine Timisoara

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Abstract: Since populational studies relying on Chronic Kidney Disease (CKD) definition, based on the decrease of glomerular filtration rate, show different conclusions regarding CKD epidemiology, our study aims to evaluate CKD using data provided by the necroptic microscopical examinations of the kidney. This method reveals pathological abnormalities of the kidney that are also relevant for CKD definition. The use of violent death cases exclusively insures a random selection of the subjects. Material and method: In order to study the kidney microscopical changes, renal tissue samples of 364 violent death cases were stained with hematoxylin-eosin and periodic acid-Schiff (PAS) for light microscopy. Results: Microscopic lesions suggested aspects which pleaded for CKD in 122 cases, representing 33.51% of all subjects. The lesions found in three cases (0.82%) might be caused by chronic glomerulonephritis. In eight cases (2.19%) renal lesions might be caused by chronic pyelonephritis (CPN). In 16 cases the lesions might plead for interstitial nephritis, different from CPN. Vascular lesions were encountered in 95 cases, representing 26.09% of all subjects. We consider that necroptic studies, especially those involving violent death cases alone, can bring significant microscopic findings and, in the same time, an important addition to the information provided by populational studies.

Key words: chronic kidney disease, violent death, necroptic study

The present study aims to evaluate the chronic kidney disease in a study group represented by all violent death cases autopsied in a given territory over a five year period. Chronic kidney disease (CKD) is defined by the presence of sustained abnormalities of renal function and results from different causes of renal injury. [1] CKD can lead to progressive loss of renal function, and may terminate in End Stage Renal Disease (ESRD) after a variable period of time, following the initiating injury. The common CKD definition relies on a functional criterion - the level of glomerular filtration rate (GFR) decrease, which lead to a five-stage classification of the disease. [2, 3]

CKD is a large and growing health care concern, but its epidemiology is not well understood. [4] Its prevalence is based on populational studies, such as the National Health
and Nutrition Examination Survey (NHANES) or the NKF Kidney Early Evaluation Program (KEEP), which suggested various CKD prevalences – 15.3% in the NHANES study and 27.1% in the KEEP study. [5, 6, 7] All the above-mentioned surveys evaluated CKD based on the level of GFR decrease.

However, most CKD patients do not progress to ESRD because they die first, the major contributor of mortality being Cardiovascular Disease (CVD). [9]

The presence of renal pathological abnormalities also represents an important diagnostic criteria for CKD. Since the forensic postmortem examination involves the microscopical examination of the kidney, we tried to evaluate CKD based on the microscopical results of violent death cases.

The use of violent death casuistry as a method for the evaluation of certain diseases has been described in literature, but has scarcely been applied to the present day. [10, 11]

Violent deaths are all deaths not caused by the spontaneous, terminal evolution of a pathological process, but resulting from an external (and usually sudden) action or force. They include homicides, suicides and accidents. [12]

This is why we consider that our method offers a populational cross-section at a certain moment in time. The results can be relevant for the general population for three reasons: to begin with - violent death affects all age groups, secondly - according to Romanian law, all violent death cases are autopsied in a legal medicine facility, and, last but not least - the fact that the only inclusion criterion is represented by the violent cause of death, without any selection regarding the preexisting pathology.

Populational studies that include necroptic microscopical examinations of the kidney are seldom cited in literature. They were prospective studies that started the follow up while the subjects were still alive. Some of the subjects deceased during the study (most of them by natural causes); part of them were autopsied, and some of the necroptic examination results were collected and analyzed. [13, 14, 15, 16, 17, 18]

**Material and method**

Between 2003 and 2007, 3915 autopsies were performed at the Legal Medicine Institute of Timisoara. Out of this total number, 2360 (60.28%) were violent deaths. We excluded 740 cases represented by: subjects under 18 years of age, deteriorated, carbonised or putrified bodies, or skeletal remains. The remaining group consists of 1620 cases of violent death in adult victims (minimum age – 18).

The forensic autopsy was performed, according to legal provisions, after at least 24 hours postmortem. We excluded the cases in which macroscopical examination revealed autolysis and putrefaction changes and used the microscopical data included in the autopsy reports regarding the examination of the kidney.

The selected study group consists of the remaining 364 cases, from which kidney samples were collected for microscopical examination.

Light microscopic study was performed. Paraffin-embedded renal tissue samples that were obtained by standard autopsy methods were cut at a 4-5 µm thickness and stained with hematoxylin-eosin and periodic acid-Schiff (PAS).

Each tissue sample was examined by two pathologists: one pathology professor from the Pathology Department of the Victor Babes University of Medicine and Pharmacy Timisoara and one forensic pathologist from the Institute of Legal Medicine Timisoara.

For each tissue sample, 70-80 glomeruli were studied, with emphasis on the percentage of glomeruli with fibrosis lesions. Interstitial lesions were analyzed concomitantly. An average of 30 renal arterioles were examined for the evaluation of vascular lesions such as
arteriolar hyalinosis and arteriolosclerosis. Larger renal vessels that appeared on some tissue specimens were also analyzed.

We mention that our study only refers to optic microscopy, without reference to the macroscopical examination of the kidney.

In violent deaths, the onset is usually instantaneous [12] and it can occur outside a medical unit. Thus, information regarding the medical history of the cases can either miss or be irrelevant. This is the reason why the only lesions we refer to in our study are those found during the forensic autopsy, in correlation with the age and gender of the subjects.

**Results**

The gender distribution shows 70.3% (256) males and 29.7% (108) females. The mean age was $55.61 \pm 10.57$ (42,86). The microscopic lesions we encountered are the following:

A. Glomerular lesions

Our study lacked any data regarding the clinical history of the autopsied subjects. The only available data were the age and gender of the subjects. These conditions influenced the classification and interpretation of glomerular lesions. The glomeruli presented primary glomerular lesions or lesions that followed the interstitium damage.

In the case of primary glomerular lesions, an acidophilic, bulky material appears, in various amounts, at glomerular level. Together with normal glomeruli, an interstitial lymphoplasmacytic infiltrate also appears, while the tubes are atrophic.

The acidophilic material tends to replace the capillary tuft with lymphoplasmacytic infiltrate and atrophic tubes almost completely.

Fibrosis and glomerular hyalinizations appear on the background of discrete lymphoplasmacytic infiltrate, along to normal and hypertrophic glomerules.

![Fig. 1 – Glomerular lesions (HE, x 100)](image1)

![Fig. 2 Chronic pyelonephritis (HE, x 200)](image2)
The arterioles show hyalinized walls and the venules have dilated lumina full of haematids. (Fig 1)

In the second case, the one of glomerular lesions which followed the interstitium damage, the lesions initially appear at the renal interstitium level starting from either the subcapsular area, or from the pyelo-calyceal area. The nephron shows some secondary changes. In these cases, the glomerular fibrosis and hyalinization is initially situated at the glomerular capsule level, namely its parietal layer. The interstitial infiltrate is rich determining, besides capsular fibrosis, tubular deformations and fibrosis.

B. Tubular lesions
As mentioned before, the epithelium of the tubes is intensely acidophilic, undefined, detached from the tubular basement membrane. The tubes are largely altered by autolysis. However, some tubular changes that accompany the glomerular ones can be distinguished. Some tubular modifications are associated with interstitial lesions.

C. Interstitial lesions
We noticed two types of interstitial lesions. In eight cases we observed interstitial lesions in the profound medulla and, sometimes, in the renal pelvis. We must mention that these interstitial lesions diminish towards the renal cortex.

They can be accompanied by tubular changes, such as significant nephrocytes atrophy, the tubular lumina dilation and hyaline cylinders that can be seen in many tubes. These elements give a thyroidization resemblance, which is usually considered characteristic for chronic pyelonephritis (CPN). (Fig. 2)

In 16 cases we encountered a triangle-shape inflammatory infiltrate, with the basis parallel to the renal capsule and the peak pointing to the renal parenchyma. The size of this formation may vary, it can be found only in the subcortical area or it can spread to the
juxtaglomerular area or even more profoundly. The infiltrate area contains lymphocytes, plasmocytes and few macrophages that replace the previous structural elements. The tubes are atrophied and, if the infiltrate also includes glomerules, they show fibrosis and hyalinization. (Fig. 3)

D. Vascular lesions

We have encountered 95 cases with vascular lesions. Knowledge of blood pressure levels of the subjects would have been extremely useful and would have allowed us to make some correlations. Within the interstitial lympho-plasmacytic infiltrate there are arterioles with hyalinized walls, as well as arterioles with thickened walls and colobated lumina.

The PAS coloration provides us with significant data referring to the type of deposits present within the arteriolar walls. The arteriolar wall thickening can be caused by accumulating of PAS-positive material, which can be found within the whole wall. (Fig. 4)

Discussions

Our approach aimed to evaluate CKD in subjects who died violently and were autopsied at the Institute of Legal Medicine Timisoara over a five year period – 2003-2007.

Unlike populational studies, this method presents the advantage of not performing any previous selection of the subjects in the study group. However, taking into account the fact that the autopsy is performed, according to the law, at least 24 h after death, the autolysis changes can influence the interpretation of the results.

Our study observed the glomerular and interstitial fibrosis lesions, the inflammatory chronic infiltrate, as well as vascular lesions represented by arteriolar hyalinosis and arteriolosclerosis.

Because in some cases these lesions can be associated, when we connected the lesions with a specific type of disease, we took into consideration the main lesion, be it glomerular, interstitial or vascular.

Microscopical lesions suggested aspects which pleaded for CKD in 122 cases. A CKD prevalence of 33.51% was the result, with a drawback represented by the fact that not all kidney lesions can be revealed due to cadaverical postmortem changes.

As mentioned before, populational studies suggested various CKD prevalences – 15.3% in the NHANES study and 27.1% in the KEEP study.

Our study established that in the three cases (0.82% of all subjects) which presented significant glomerular fibrosis, the lesions might have been caused by chronic glomerulonephritis (CGN). Lesions of glomerulosclerosis and hyalinosis accompanied by an interstitial chronic inflammatory infiltrate might plead for this diagnosis. In one case they were associated with interstitial fibrosis.

Glomerulosclerosis lesions are encountered in diffuse glomerulonephritis, when all the glomeruli are affected, or in advanced stages of focal segmental glomerulonephritis. There were no cases of focal segmental glomerulonephritis in our subjects. Our three cases showed diffuse glomerular lesions, which affected more than 50% of the glomeruli on each tissue sample.

The other types of glomerulonephritis with various glomerular lesions cannot be diagnosed because of autolitical changes.

Because the necroptical microscopic examination can define the above type of lesions, its importance might mainly reside in underlining the presence of lesions encountered in advanced stages of CKD.

It can be mentioned that in chronic glomerulonephritis the glomerular fibrosis process is accompanied by tubulointerstitial lesions, which were present in our cases as well.
We encountered 95 cases of vascular lesions (26.09% of all subjects). We noticed no difference between renal arteriolar changes and those of larger renal vessels, so the vascular lesions we encountered will be presented globally.

The presence of arteriolar vascular lesions can be related with pathological vascular alterations which affect the kidneys.

The main disease which determines renal vascular lesions is nephroangiosclerosis following arterial hypertension (AH). AH is a frequent disease whose prevalence increases with age. Approximately 25% of the general population is considered to be affected by AH.[20] Tracy et al. mention that both hyalinizations of arterioles and arteriosclerosis tend to increase with age in most visceral organs, both features are exaggerated in the kidneys of hypertensive compared with nonhypertensive subjects.[21]

Renal vascular lesions prevalence, noticed by us after interpreting the results of the renal microcopical necroptical examination, matches the AH prevalence reported by populational studies.

Diabetes is another cause of arteriolar renal lesions. Diabetics tend to develop renal arteriolar hyalinization.[22] Unfortunately, we didn’t have any data regarding history of diabetes for our subjects. Other authors reported that cigarette smokers may have excessive microvascular abnormalities in the kidney and other viscera.[23]

While advancing, atherosclerosis may produce nephroangiosclerosis besides large vessels lesions. The atherosclerotic process progressively affects the vessels in other organs, mainly the heart and brain - well vascularised organs that receive a significant amount from the cardiac output, as do the kidneys.

In a previous study, we reported the presence of coronary atherosclerotic lesions at a significant percentage from the necroptical examinations included in that study.[24] Correlations between these vascular lesions and the renal ones are not part of this approach.

In eight cases we noticed an abundant inflammatory infiltrate in the deep renal medulla, and sometimes in the renal pelvis. The intensity of the interstitial lesions decreases towards the renal cortex. The cause might be CPN. We must mention that in three of the cases, these lesions were accompanied by interstitial fibrosis.

In practice, CPN is more frequent than CGN, which is also revealed by our study – 2.19% CPN as compared to 0.82% CGN.

At the same time, in 16 cases (other than the eight cases of CPN) we noticed interstitial lesions where the inflammatory infiltrate was triangle-shaped, with the basis parallel to the renal capsule and the peak pointing to the renal parenchyma, or the pelvis respectively. The size of this formation may vary, it can be found only in the renal cortex or it can spread more profoundly. Some glomeruli with fibrosis and hyalinizations can be found in these infiltrates.

We don’t know the significance of these lesions. Being placed in the cortex, they differ from the lesions caused by CPN. They might plead for interstitial nephritis, different from CPN.

Some authors related the interstitial lesions found in the renal cortex interstitium to phenacetin nephritis. Thus, Robbins states that in phenacetin nephritis papillary necrosis occurs first, and cortical tubulointerstitial nephritis is a secondary phenomenon.[25]

Lacking any data about an ante-mortem phenacetin use or any other etiological factor related to interstitial nephritis, we cannot establish any other causing factor for interstitial nephropathy that might induce these microscopical changes.
Conclusions
Our study analyzes the renal microcopical lesions found in a group of 364 violent death cases.

It reports microcopical changes which are not affected by autolysis during the first 24 h after death, because the medico-legal autopsy is performed, according to legal provisions, after this period of time.

The occurence of glomerular and interstitial fibrosis, as well as the interstitial inflammatory infiltrate and vascular lesions reflect the presence of CKD.

CKD lesions identified by microscopical necroptic examination reveal its prevalence in a populational group at a certain moment. The encountered data must be related to populational studies regarding CKD epidemiology. In our case, they refer to NHANES and KEEP results.

The drawback of the method suggested by us is the possibility of revealing only the glomerular lesions in advanced stages of renal diseases, when glomerular fibrosis lesions occur, except for focal segmental glomerulonephritis cases.

Vascular lesions found at necroptic microscopical examination are also related to those mentioned in populational studies.

Our approach also underlines the interstitial lesions, some of which might be caused by CPN, while others remain of unknown etiology, although we suspect an interstitial nephritis other than CPN, possibly phenacetin nephritis.

The macroscopic examination of the kidneys, although it is not the aim of the present study, showed three cases of polycystic kidney disease. They may also be considered CKD cases.

We believe that, since renal microscopical lesions reflect CKD, the use of microscopic renal necroptical examinations might offer a clear image of CKD prevalence in a populational group at a certain moment, with the advantage of cross-sectioning.

Populational studies usually manage to convey a broad image regarding a given pathology, but few of them also rely on data provided by microscopical examinations.

The analysis of data provided by the autopsy reports in violent death cases can provide histopathological information, further to those obtained from populational studies. Even more so, the employment of violent death cases only, has the advantage of not making any subject selection.

References