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Electron microscopy situation in the diagnosis of minimal change disease; a 16-year survey

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ABSTRACT

Introduction: Minimal change disease (MCD) is one of the most common diseases affecting glomeruli and causing nephrotic syndrome in both adults and children.
Objectives: The aim of this study was to evaluate the degree of accordance between light, fluorescent, and electron microscopies in the diagnosis of MCD.
Patients and Methods: In this cross-sectional study, we investigated kidney biopsies which were sent to the referral electron microscopy unit (affiliated to Shiraz University of Medical Sciences) from 2001 to 2016. The final diagnosis of MCD was based on the electron microscopy (EM) study. For each patient, the primary light microscopy (LM) finding was compared with EM diagnosis. The available patients' demographic, clinical, and paraclinical data were extracted. All the statistical analysis was performed in SPSS 19.0 and $P<0.05$ was considered as statistically significant.
Results: Among all 2865 kidney biopsies, the data of 143 (5.0%) patients with approved MCD by EM were analyzed. The mean age of patients was 23.1± 17.4 years since most of them were male (54.9%). Normal blood urea nitrogen (BUN) and creatinine were observed in 76.6% and 78.6% of them respectively. Around, 140 (97.9%) of patients had no tubular atrophy and interstitial fibrosis. The role of EM in the diagnosis of MCD for 61 (50.0%) of cases were essential, for 61 (50.0%) cases were helpful while there was no case with the role of non-necessary. The immunofluorescence (IF) study was performed for 99 (69.2%) patients. Among them, it was negative for 60 (60.6%) of cases and was positive for IgM and C3 in 19 (19.2%) and 11 (11.1%) of cases respectively. The proportion of flank pain was significantly higher among adults.
Conclusion: The importance of EM for the diagnosis of MCD is indispensable and undeniable; while LM is not capable of independently leading to a certain diagnosis of MCD. Considering the limitations of using EM, the results obtained from this study can help with the appropriate use of electron microscopy and help physicians to reach earlier diagnosed.

Implication for health policy/practice/research/medical education:
Among pathologic diagnostic tools for better diagnosis of minimal change disease, electron microscopy plays an important role. The results of our study give a clue to clinicians and nephropathologists to choose the best diagnostic method for each patient.
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Original

Introduction

Minimal change disease (MCD) is known as one of the illnesses affecting glomerulus and causing nephrotic syndrome (1). In adults, MCD is responsible for 10 to 15% of idiopathic nephrotic syndromes (2). In children, MCD is the most common cause of nephrotic syndrome (3). Epidemiologically, the prevalence of MCD among children is estimated between 2 to 7 individuals per 100 000 patients (4). Also, in children the involvement ratio of boys to girls is double; however, there is no gender ratio in adults (5). The most common clinical manifestations of the disease are edema around the eyes, lower limbs, scrotum, and labia (6). In some cases, the onset of symptoms such as edema and proteinuria may result from taking certain

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medications such as nonsteroidal anti-inflammatory drugs (NSAIDs).

The pathogenesis of MCD is not yet fully understood, since T lymphocytes appear to be responsible for glomerular degradation and symptom production. It has been observed that during the recurrence of the disease, CD8 lymphocytes increased and CD4 lymphocytes decreased. The growth of CD8 cells strongly supports the role of T cells in the pathogenesis of the disease (7). Of all Th2-secreting cytokines, IL13 appears to play a more important role in the elimination and destroying of podocytes (8). Acute renal failure is not a common complication in MCD. People with higher ages, high systolic blood pressure, and atherosclerotic vascular changes are more likely to be exposed to acute renal failure due to renal artery involvement (9). The first line of treatment for MCD is steroids that have different responses in children and adults (10).

The prognosis of MCD in children is more favorable than that of adults, while 70% of children suffering from MCD enter adulthood with no renal insufficiency and no urinary problems. The course is also very good in adults, with a rate of above 90% for 10-year survival, without moving to end-stage renal disease (11).

Few morphologic findings exist in light microscopy (LM) of MCD. Besides, there are no specific findings by immunofluorescence (IF) microscopy except small amounts of C3 or IgM depositions. However, effacement and flattening of foot processes of podocytes are characteristics for MCD (12,13). In fact, the destruction of the epithelium layer and the disappearance of the foot processes, holes, and fine pores between them, which are the main pathology in MCD, can only be observed by electron microscopy (EM) (14). Therefore, if we evaluate the specimen with only light microscopy, the diagnosis can easily miss (15). This misdiagnosis may also occur for other diseases that are detectable only by EM study in the early stages (16).

The use of EM has limitations such as complex preparation process, specific techniques, high cost, and lack of easy accessibility, its applications in pathology today are limited to specific cases (17). However, due to the capabilities of EM in kidney biopsy tissues, such as the significant magnification of the glomerular basement membrane or the ability to detect immune complexes, kidney biopsy specimens continue to be one of the common uses of EM in conjunction with IF and LM during the last decades (16). Some researchers have shown that in 85% of kidney biopsies, EM has been used (18). Routine use of EM has also been shown to be useful in the diagnosis and classification of glomerular kidney diseases particularly MCD (19).

Objectives

The aim of this study was to evaluate the degree of accordance between light, fluorescent and electron microscopies in the diagnosis of MCD and to assess the morphologic changes of the disease in toluidine blue staining as well as clinical and paraclinical findings.

Patients and Methods

Study design

In this cross-sectional study, we examined the biopsy specimens of all patients from Namazi, Faghihi, Shiraz, Hafez, and Dena hospitals referred to the EM Unit (affiliated to Shiraz University of Medical Sciences) from 2001 to 2016. All patients whose MCD diagnosis was confirmed by EM were included in this study. Samples from kidney transplanted patients and samples that did not contain renal cortex (due to the poor technique of kidney biopsy) were excluded from the study. The fresh biopsy tissue from the kidney was cut into pieces for preparation of LM and EM. For LM after formalin fixation and paraffin embedding, sections of 2-3 µm were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Jones methenamine silver, and Masson's trichrome. Each fresh specimen was cut into small blocks and the specimens were prepared for examination under an EM. Pieces of tissue were cut into 1 µm in diameter and stained with toluene blue to see glomeruli under LM. Then, the grid was examined under an EM. For each sample, the LM and EM data along with clinical and paraclinical data were statistically analyzed. Immediately after the kidney biopsy, direct IF experiments were performed on 3-4 µm cyanoacetate sections using fluorescein isothiocyanate conjugated with antibodies to detect IgG, IgA, IgM, C3, and C1q. All biopsies were examined by LM before definitive diagnosis by EM. For each patient, the initial diagnosis by LM was compared with the EM diagnosis. Finally, similar to previous studies (28,29), the role of EM in the final diagnosis was divided into three subgroups;

1. Essential; LM diagnosis is different from EM diagnosis.
2. Helpful; LM diagnosis was consistent with the EM but was not definitive, and it was expressed as a possibility. EM was still required to confirm and rule out other diagnoses.
3. Non-necessary; cases where the LM had the same certainty as to the diagnosis of an EM.

All pathology request sheets and patient records were included, involving demographic information of patients with MCD as well as clinical and paraclinical information. In this study, we compared the clinical findings with paraclinical ones considering the following definitions;

- Hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or ongoing treatment with antihypertensive medications or based on patient history and medical documents)
- Hematuria (including both gross and microscopic [over three-plus spot urine red blood cell (RBC) or two-plus spot urine blood)]
- Proteinuria (over 150 mg/24 h urine protein or over

two-plus spot urine protein when 24-hour urine collection was not performed).

Statistical analysis

Descriptive statistics were reported as frequency (%) or mean ± SD for qualitative and quantitative variables, respectively. Accordingly, to investigate the difference between two groups, we used an independent *t* test. Statistical bivariate associations were examined using chi-square or Fisher's exact test in SPSS 19.0, and $P<0.05$ was considered as statistically significant.

Results

Among all 2865 kidney biopsies, data of 143 (5.0%) patients with approved MCD in EM were analyzed in the current study. The mean age of the patients was 23.1± 17.4 years since most of them were male (54.9%). Normal blood urea nitrogen (BUN) and creatinine were observed in 76.6% and 78.6% of them respectively. Around, 140 (97.9%) patients had no tubular atrophy and interstitial fibrosis in EM, while in LM 116 (81.1%) and 128 (89.5%) of patients had no tubular atrophy and interstitial fibrosis, respectively. Moreover, the number of observed glomeruli was 7.52±4.3, 15.0±10.2 in EM and LM, respectively (Table 1). EM study was recommended in LM reports of 42.7% patients for better evaluation.

Out of 143 MCD patients, 62 (43.3%) were children and 81 (56.7%) were adults. There was no significant difference between children and adults except for flank pain. Comparison of the mean number of glomeruli between children and adults revealed that the mean of glomeruli among children was significantly more in EM (8.8 versus 6.6, $P=0.001$), LM (18.0 versus 13.7, $P=0.001$) and IF (7.7 versus 4.4, $P=0.002$; Table 1).

Out of all 143 cases, the IF study was performed for 99 (69.2%) patients. Among them, it was negative for antibody deposits in 60 (60.6%) cases and was positive for IgM and C3 in 19 (19.2%) cases and 11 (11.1%) cases respectively (Figure 1).

The role of EM in the diagnosis of MCD for 61 cases (50.0%) was essential, while for 61 (50.0%) cases it was helpful since there was no case with the role of non-necessary. The most frequent misdiagnosis or concurrent pathology in LM was mesangial proliferation in 23 cases followed by focal segmental glomerulosclerosis (FSGS) in 8 cases (Table 2).

Table 1. Association between the characteristics under the study with age and gender

Variables		Age				Gender			
		Children (n=62)	Adult (n=81)	Total	*P* value	Male (n=78)	Female (n=64)	Total	*P* value
Edema	No	5 (13.5)	9 (13.0)	14 (13.2)	0.82	8 (13.3)	6 (13.0)	14 (13.2)	0.97
	Yes	32 (86.5)	60 (87.0)	92 (86.8)		52 (86.7)	40 (87.0)	92 (86.8)	
Hematuria	No	31 (83.8)	48 (82.8)	79 (83.2)	0.90	45 (83.3)	34 (82.9)	79 (83.2)	0.96
	Yes	6 (16.2)	10 (17.2)	16 (16.8)		9 (16.7)	7 (17.1)	16 (16.8)	
Proteinuria	No	12 (19.4)	12 (14.8)	24 (16.8)	0.47	13 (16.7)	10 (15.6)	23 (16.2)	0.95
	Yes	50 (80.6)	69 (85.2)	119 (83.2)		65 (83.3)	54 (84.4)	119 (83.8)	
Flank pain	No	31 (93.2)	41 (70.7)	72 (79.1)	**0.01**	42 (82.4)	30 (75.0)	72 (79.1)	0.39
	Yes	2 (6.1)	17 (29.3)	19 (20.9)		9 (17.6)	10 (25.0)	19 (20.9)	
HTN	No	22 (73.3)	38 (76.0)	60 (75.0)	0.79	33 (71.7)	27 (79.4)	60 (75.0)	0.43
	Yes	8 (26.7)	12 (24.0)	20 (25.0)		13 (28.3)	7 (20.6)	20 (25.0)	
Increased serum BUN	No	30 (81.1)	52 (74.3)	82 (76.6)	0.72	41 (68.3)	41 (87.2)	82 (76.6)	0.07
	Yes	7 (18.9)	18 (25.7)	25 (23.4)		19(31.7)	6 (12.8)	25 (23.4)	
Increased serum creatinine	No	28 (82.4)	53 (76.8)	81 (78.6)	0.15	43 (74.6)	38 (74.4)	81 (78.6)	0.44
	Yes	6 (17.6)	16 (23.2)	22 (21.4)		15 (25.9)	7 (15.6)	22 (21.4)	
Tubular atrophy EM	No	62 (100)	78 (96.3)	140 (97.9)	0.26	76 (97.4)	63 (98.4)	139 (97.9)	0.99
	Mild	0 (0)	3 (3.7)	3 (2.1)		2 (2.6)	1 (1.6)	3 (2.1)	
Interstitial Fibrosis EM	No	62 (100)	78 (96.3)	140 (97.9)	0.26	76 (97.4)	63 (98.4)	139 (97.9)	0.99
	Mild	0 (0)	3 (3.7)	3 (2.1)		2 (2.6)	1 (1.6)	3 (2.1)	
Tubular atrophy LM	No	50 (80.6)	66 (81.5)	116 (81.1)	0.99	63 (80.8)	52 (81.3)	115 (81.0)	0.58
	Mild	11 (17.7)	14 (17.3)	25 (17.5)		13 (16.7)	12 (18.8)	25 (17.6)	
	Moderate	1 (1.6)	1 (1.2)	2 (1.4)		2 (2.6)	0 (0)	2 (1.4)	
Interstitial Fibrosis LM	No	57 (91.9)	71 (87.7)	128 (89.5)	0.26	70 (89.7)	57 (89.1)	127 (89.4)	0.77
	Mild	4 (6.5)	10 (12.3)	14 (9.8)		8 (10.3)	6 (9.4)	14 (9.9)	
	Severe	1 (1.6)	0 (0)	1 (0.7)		0 (0)	1 (1.6)	1 (0.7)	
IF	Negative	27 (60.0)	33 (61.1)	60 (60.6)	0.99	31 (57.4)	28 (63.6)	59 (60.2)	0.54
	Positive	18 (40.0)	21 (38.9)	39 (39.4)		23 (42.6)	16 (36.4)	39 (39.8)	
EM-Number of glomeruli		8.84±5.0	6.62±3.2	7.52±4.3	**0.001**	7.8±4.1	7.2±4.5	7.5±4.3	0.40
LM- Number of glomeruli		19.3±11.8	13.4±7.8	15.0±10.2	**0.001**	18.0±10.3	13.7±9.7	16.0±10.2	**0.01**
IF- Number of glomeruli		7.7±6.9	4.4±3.3	5.9±5.4	**0.002**	6.3±5.6	5.4±5.2	5.9±5.4	0.47
Mean BP		89.1±12.1	95.0±8.8	92.3±10.4	**0.01**	93.3±11.1	92.4±9.6	92.3±10.4	0.69

LM; Light microscopy, EM; Electron microscopy, IF; Immunofluorescence microscopy, BP; Blood pressure, HTN; Hypertension, BUN; Blood urea nitrogen.

LM examination of the kidney biopsies of all the patients stained with H&E, PAS, Jones methenamine silver, and Masson's trichrome showed no visible changes except for mild mesangial proliferation in some cases. Plastic sections with 1 µm thickness stained with toluidine blue studied under LM showed uniform flattening of the foot processes of the podocytes with few visible vacuolizations (Figure 2). Ultrastructural examination of glomeruli of all the patients showed characteristic diffuse and extensive effacement of the foot processes of the visceral epithelial cells (podocytes), as shown in Figure 3. Electron dense deposits were not identified around the basement membrane of the capillary wall. The thickness of the basement membrane appeared normal. Electron micrographs of normal foot processes of the podocytes are shown in Figure 4 for better comparison.

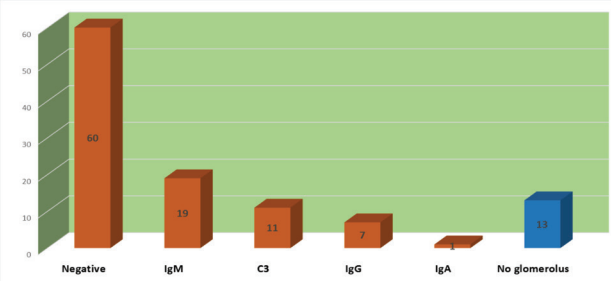

Figure 1. Frequency of immunofluorescence microscopy study of patients with minimal change disease.

Discussion

In this study, we evaluated the clinical and para-clinical information of patients who had undergone renal biopsy between 2001 and 2016 and were confirmed by a transmission electron microscope for the diagnosis of MCD. According to the authors' investigations, this study is one of the largest studies of MCD conducted via EM in Iran.

We found that of the 2865 total kidney biopsies sent to the EM unit over the past 16 years, 143 patients (5% of total kidney samples) were diagnosed as MCD. The mean age of the patients with MCD was 23.1 ± 17.4 years and most of them were male. In total, 86.6% had edema, 83.2% had proteinuria and 16.8% had hematuria. The proportion of flank pain was significantly higher among adults. The mean number of glomeruli in children was significantly higher in all three types of EM, LM and IF microscopies. Out of all 143 cases, the IF study was performed for 99 (69.2%) patients. Among them, it was negative for antibody deposits in 60 (60.6%) cases and was positive for IgM and C3 in 19 (19.2%) and 11 (11.1%) cases respectively. The role of EM in the diagnosis of MCD in 61 (50.0%) cases

Table 2. Role of electron microscopy in the diagnosis of minimal change disease

Role of electron microscopy in the diagnosis	Light microscopic findings		No. of patients (%)
1-Non-necessary	Definite diagnosis of MCD		0 (0)
2-Helpful	Suggestive of (not definite)	Only MCD	40 (32.8)
		MCD + Mesangial proliferation	14 (11.5)
		MCD + Acute tubulointerstitial nephritis	5 (4.1)
		MCD + Acute and chronic tubulointerstitial nephritis	1 (0.8)
		MCD + Benign nephrosclerosis	1 (0.8)
Subtotal			**61 (50.0)**
3-Essential	Mesangial proliferation		23 (18.9)
	FSGS		8 (5.6)
	No specific pathologic change (normal)		4 (3.3)
	MGN		4 (3.3)
	Acute tubulointerstitial nephritis		3 (2.5)
	Mesangial proliferation + FSGS		3 (2.5)
	MPGN		1 (0.8)
	Mesangial proliferation + crescent formation		1 (0.8)
	Mesangial proliferation + congenital nephrotic syndrome		1 (0.8)
	Mesangial proliferation + IgA nephropathy		1 (0.8)
	Global glomerulosclerosis + chronic tubulointerstitial nephritis		1 (0.8)
	Acute and chronic tubulointerstitial nephritis		1 (0.8)
Subtotal			**61 (50.0)**
Total			122 (100)
	Insufficient tissue for LM diagnosis		14
	LM did not do for them		7

MCD: Minimal change disease; FSGS: Focal segmental glomerulosclerosis; MGN: Membranous glomerulonephritis; MPGN: Membranoproliferative glomerulonephritis.

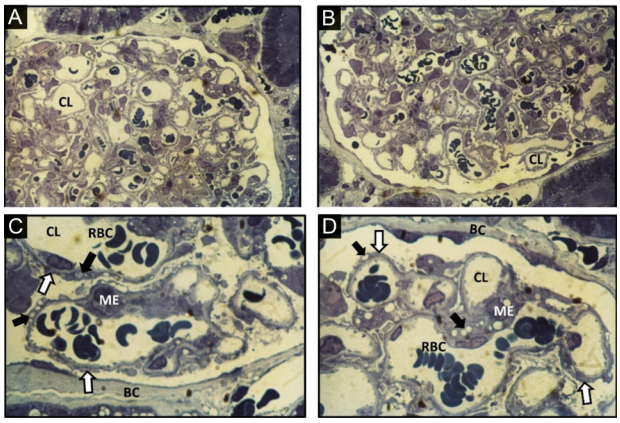

Figure 2. Plastic sections with 1 µm thickness stained with toluidine blue studied under light microscopy show uniform flattening of the foot process of the podocytes (white arrow). Some of them contain few vacuoles (black arrow) which are characteristics of minimal change disease. The capillary lumen contains RBCs and capillary walls thicknesses are normal. (**A** and **B** ×400, **C** and **D** ×1000). RBC: Red blood cells, BC: Bowman's capsule, ME: Mesangial areas.

was necessary; 61 (50.0%) cases were helpful, and its role was not unnecessary in any case.

Our study showed that out of 2865 total kidney biopsies, 143 patients were diagnosed as MCD (5.0% of total kidney samples). However, in a five-year study accomplished by Rathi et al, out of 364 kidney samples, 48 (about 13.1%) were diagnosed with MCD (31). In another large study conducted by Polito et al in Brazil in 2009, from 4619 kidney samples with an initial diagnosis of glomerulonephritis, MCD with 15.5% after FSGS, membranous nephropathy, and IgA nephropathy, was the most common diagnoses (32). This indicates that in recent studies, the percentage of MCD is higher than our study; which may reflect the different distribution of the disease in different geographical areas and the lower percentage of the disease in our region.

The mean age of the patients in the study was 23.1± 17.4 years and most of them were men (54.9%). According to the study by Keskar et al which was conducted on 61 patients with MCD, the mean age of the patients was 30.46 ± 13.43 years and most of them were male (54.09%) (20). These findings are in line with our study. Another recent study by Han et al at the Guangdong Medical University in 2020 reported a mean age of 27.7 ± 13.3 years for patients diagnosed with idiopathic MCD (21). The mean age of the participants in another study conducted by Huang et al (Taiwan) was reported 30.9±16.1 years (22).

In our study, of 143 patients with MCD, 62 (43.3%) patients were pediatric and 81 (56.7%) patients were adults. In a retrospective study executed by Das et al, out of 279 patients with MCD, 30.1% were pediatric and 69.9% were adults (24). It indicates that the number of adults with MCD may be greater than children, which is consistent with our study.

In our study, only 2.1% of patients had tubular atrophy

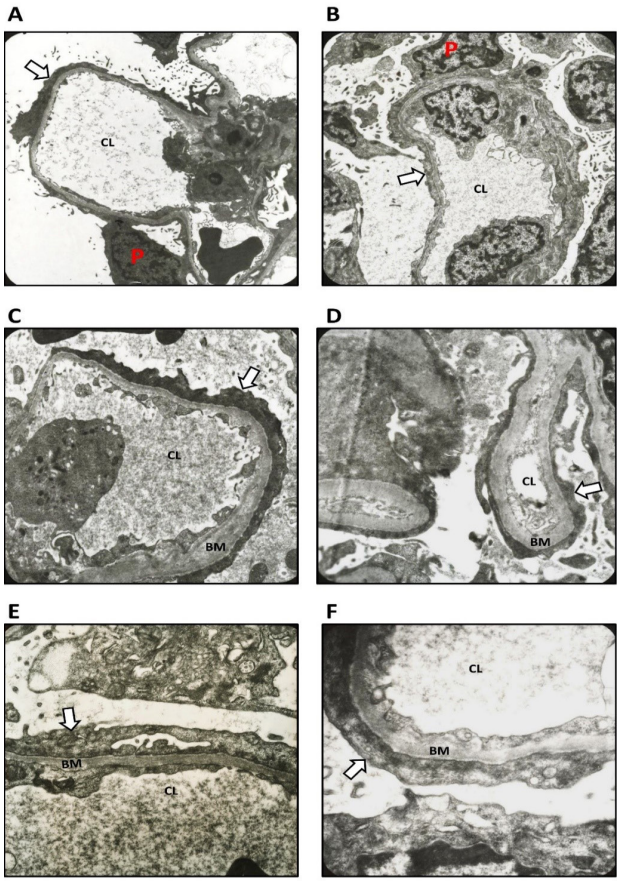

Figure 3. Electron micrographs of a portion of the glomerulus in different patients with minimal change disease show diffuse extensive effacement of foot processes of podocytes (white arrow). (Uranyl acetate and lead citrate. **A**×3597, **B** ×4646, **C** ×7750, **D** ×10000, **E** ×12930, **F** ×21560). CL: Capillary lumen, BM: Basement membrane, P: Podocyte.

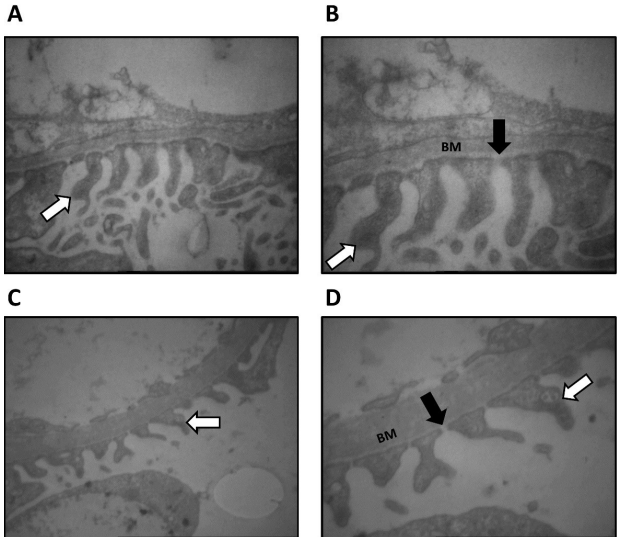

Figure 4. Electron micrographs of normal Glomeruli (control) show capillary wall with normal foot processes of the podocytes (white arrow), well visualized by small slit gap between them (black arrow). The basement membrane (BM) thickness is normal without any deposits. (Uranyl acetate and lead citrate. **A** and **C** ×10000, **B** and **D** ×16700).

and interstitial fibrosis in EM. In a study carried out by Keskar et al in 2013 in India, only 6% out of 61 cases had tubular atrophy and interstitial fibrosis, and the rest (94%) had no tubular atrophy and interstitial fibrosis (20). In another study conducted in France in 2018 on 656 cases of MCD, only 13 cases (1.9%) had tubular atrophy and interstitial fibrosis (98.1% had no fibrosis and atrophy) (23). The results of these two studies are in line with the findings of our research. This low rate of tubular atrophy and interstitial fibrosis is due to the pathogenesis of the disease while pathologic changes are only partially seen in the podocytes (20). On the other hand, these two microscopic factors, tubular atrophy and interstitial fibrosis, somehow indicate disease progression and poor prognosis in kidney diseases; hence, their low incidence in MCD indicates a good course and a favorable prognosis.

Additionally, our study showed that the mean number of glomeruli was 7.52±4.3in EM, 15.0±10.2 in LM, and 5.9±5.4 in IF microscopy. Also, the mean number of glomeruli in children was significantly higher than an adult in all three EM ($P=0.001$), LM ($P=0.001$), and IF microscopy ($P=0.002$). In a study carried out in 2008 in Nepal, out of 137 kidney biopsies assessed with LM, 67.9% had at least 6 glomeruli, 17.5% had between 3 to 5 glomeruli, and 14.6% had between 1 and 2 glomeruli (25). In another study by Golay et al in India, the mean number of glomeruli in the kidney samples using LM was 28.84±14.62 (26). The results of these studies are nearly consistent with our study. It also appears that the high number of glomeruli in children is due to their smaller kidneys and the greater number of glomeruli per volume unit, which is an expected finding.

We found that the role of EM in the diagnosis of MCD was necessary in half of the cases, helpful in another half of cases, and unnecessary in no case. In a study accomplished by Rivera et al in the USA in 48 cases, the role of electron microscopy was necessary for the diagnosis of MCD in 73% of cases and helpful in 27% of cases (27). The results of this study were in line with our study. Likewise, in another study in India in 2016 conducted on 18 cases of MCD, the role of EM was necessary for diagnosis in 11% of cases, and in 89% was helpful (28). Furthermore, in a study by Zuppan in California which was conducted on 80 patients with the diagnosis of renal disease, the role of EM in the diagnosis of glomerular disease among children was necessary in 50 cases (63%), helpful in18 cases (23%), and unnecessary for only 9 cases (11%). In this study, out of the 6 cases diagnosed with MCD, EM was necessary for diagnosis in all 6 cases (100%) (29).

In the present study, the IF study was performed for 99 patients (69.2% of all patients). Among them, it was negative for 60 (60.6%) cases and was positive for IgM, C3, IgG, and IgA in 19, 11, 7, and 1 cases, respectively. In a study by Huang et al in Taiwan on 46 cases of MCD, only 23.9% of them had positive IF findings. They found that IgM with 8 cases was the most frequent followed by IgA, C3, and IgG, which is different from our study (22). Herlitz et al in a retrospective cohort study on 17 patients with IgA nephropathy presented with nephrotic range proteinuria showed that they had also superimposed MCD (30). This result can demonstrate the association between MCD and IgA nephropathy. However, our study does not support this hypothesis. Further studies with larger sample sizes are recommended for future investigations on this issue.

In this study, we also found that mesangial proliferation followed by FSGS were the most common misdiagnosis in LM. A study that was done on 17 patients with both MCD and IgA nephropathy indicated that 82.4% of them had mesangioproliferative changes in LM (30). This finding represents that MCD has associated with mesangioproliferative changes. In fact, it seems that mesangioproliferative changes in LM are mostly a sign of association between these two diseases (not misdiagnosis). In another study in 2018 in the UK on MCD patients, the most misdiagnosis in LM was FSGS with 55% (33). However, in our study, the FSGS misdiagnosis was 8.6% which is lower than the mentioned study.

In this study, 1-µm thickness sections stained with toluidine blue demonstrated a valuable clue for the diagnosis of MCD. Therefore, we suggest, in addition to routine staining of the kidney tissues for light microscopy, the plastic-section stained with toluidine blue could be helpful for better diagnosis.

Conclusion

The importance of EM for the diagnosis of MCD is indispensable and undeniable, since, the LM is not capable of independently leading to a certain diagnosis of MCD. Considering the limitations of using EM (such as being an expensive and time-consuming process), the results obtained from this study can help with the appropriate use of electron microscopy and aid physicians to reach an earlier diagnosis.

Moreover, we found that toluidine blue staining on 1 µm sections from resin blocks provides useful information for the diagnosis of MCD.

Limitations of the study

One limitation of this study was due to retrospective data collection. Some incomplete medical charts of patients led to missing some information. Another limitation is the lack of access to some patients due to their poor follow-ups. It is recommended that researchers use a larger statistical population for future studies to make the results more conclusive.

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Authors' contribution

Conceptualization: SMO. Methodology: SMO, SHO, AS. Validation: SMO. Formal Analysis: HRS. Investigation: AS, SHO, SAHZ. Resources: SMO. Data Curation: SHO, HRS. Writing—Original Draft Preparation: SAHZ, SHO. Writing—Review and Editing: SMO, AS, HRS. Visualization: SMO. Supervision: SMO. Project Administration: SMO. Funding Acquisition: SMO.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical issues

The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the patients. This study was approved by the ethics committee of Shiraz University of Medical Sciences (#IR.sums.med.rec.1397.217). Besides, this study was extracted from the M.D, thesis of Alireza Shahriari at this university (proposal #1396-01-01-14987). Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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