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EARLY EFFECTS OF HIGH CHOLESTEROL DIET ON THE KIDNEY OF AN ANIMAL MODEL

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Abstract

BACKGROUND: Previous studies have proven that there exists a complex association between progressive kidney damage and hypercholesterolemia. Most of them focused on the impact of chronically high blood cholesterol levels on the kidney. Information on the early effects of hypercholesterolemia on the kidney is still lacking. The aim of this study is therefore to determine early effects of high cholesterol diet on the kidney in an animal model.

METHODOLOGY: Ten female Sprague-Dawley rats were divided into two groups: the control group, fed with commercial rat pellet and the high cholesterol diet (HCD) group, fed with 12% cholesterol diet with 0.3% cholic acid. Biochemical analysis of the lipid profile and renal function were performed at completed 48 hours, 7 days, and 6 weeks of the experiment. The animals were sacrificed at 6 weeks and the kidneys were harvested for histological examination.

RESULTS: The HCD group rats had significantly higher levels of serum total cholesterol (at 7 days and 6 weeks). The HDL-c and triglyceride levels were, however lower at 6 weeks. The mean serum creatinine level of the HCD group was increased after 48 hours and 7 days compared to control group. Histological examination of the kidney tissue of the HCD group at 6 weeks revealed segmental mesangial hypercellularity and mesangial matrix expansion of the glomeruli.

CONCLUSION: The 12% cholesterol diet induced dyslipidaemia in the animal model. It resulted in acute kidney injury based on the serum creatinine at 48 hours and also 7 days. Kidney tissues examined at 6 weeks revealed changes confined to mesangial cells of the renal glomeruli.

Key words: High Cholesterol Diet, serum creatinine

Introduction

Kidney diseases include acute kidney injury (AKI), sub-acute kidney injury, and chronic kidney disease. AKI is characterized by rapid and sometimes fatal loss of kidney function, leading to inability to maintain body fluids, electrolytes and acid-base homeostasis, and causing accumulation of end products of nitrogen metabolism (urea) and creatinine, or reduction in urine output, or both (Bellomo, Kellum, & Ronco, 2012).

Acute kidney injury (AKI) can be divided into pre-renal AKI which is caused by a reduction in renal perfusion pressure without damage to the renal parenchyma while post-renal AKI is characterized by obstruction to urinary flow (Fauci et al., 2008). Renal causes AKI include severe or prolonged decrease in renal perfusion, nephrotoxicity, severe acute glomerulonephritis, acute interstitial nephritis, and injury to the intra-renal vessels (Basile, Anderson, & Sutton, 2012).

Several animal models have been used to study the AKI, such as Ischemia-reperfusion-induced AKI (Bhalodia et al., 2009), Glycerol-induced AKI (Karam et al., 1995), Gentamicin-induced AKI (Razik, Farrag, Ibrahim, & El-Sayed, 2016), Cisplatin-induced AKI (Yuki Izuwa, Jun-ichi Kusaba & Tetsuya Aiba, 2009), Radiocontrast media-induced AKI (Erley et al., 1997), Folic acid-induced AKI (Wan et al., 2006), Osmosis-induced AKI (Duarte, 1999), and many other models (Singh et al., 2012).

The incidence of acute kidney diseases and disorders are on the rise in both developed and developing countries (Lameire et al., 2013). It is associated with severe complications where it increases the risk of short-term and long-term mortality, development of chronic kidney disease and rapid progression to end-stage renal disease (Rewa & Bagshaw, 2014). It has been shown that diet and lifestyle have important roles in its development. High cholesterol diet has been documented to cause elevation of blood pressure and induce kidney injury (Al-Rejaie, Abuohashish, Alkhamees, Aleisa, & Alroujayee, 2012). Researchers have proven that there exists a complex association between progressive kidney damage and hypercholesterolemia (Ghada, 2014). These findings are of concerns as approximately 50% of the middle-aged adult population have been shown to have total cholesterol levels above the normal range (Chade et al., 2005). Most previous studies have focused on the impact of chronically high blood cholesterol levels on kidney and have documented the development of focal glomerulosclerosis and proteinuria that rapidly progressed to renal failure (Deepa & Varalakshmi, 2006). There is however minimal information available in the literature with regard to early (acute and subacute) effects of hypercholesterolemia on the kidney (Abdel-Hafez, Othman, & Seleim, 2011). Hence the objective of this study was to determine the early effects of high cholesterol diet on the kidney.

Materials and Methods

Animals

Ten female Sprague-Dawley rats (age 6- 8 weeks) weighing 140-170 grams were used in this study. The rats were purchased from A-Sapphire Enterprise, Seri Kembangan, Selangor. Each cage housed two rats under standard experimental conditions of 20- 26°C at 50- 70% humidity with 12 hours light/dark cycles. They had free access of water and food throughout the experiment. The animal handling procedures, treatment and experimental protocols were approved by the Institutional Animal Care and Use Committee, International Islamic University Malaysia [IIUM / IACUC Approval / 2016/12/83].

High cholesterol diet

Twelve percent cholesterol diet was prepared by mixing of 1kg of commercial rat pellet in powder form with 120 grams of analytical pure cholesterol powder (Nacalai-Tesque, KYOTO, JAPAN. Lot No. M4T5494. Code 08721-75) and 3 grams of cholic acid (Nacalai-Tesque, KYOTO, JAPAN. Lot No. M6H9123. Code 08805-56) in order to produce stable hypercholesterolemia (Monte & Jimenez, 1993). The preparation was carried out weekly to avoid oxidative modification of the cholesterol.

Experimental design

After 10 days of acclimatization, the rats were randomly divided into two groups. Group I served as the control group (n=5) and was fed with commercial rat pellet. Group II served as the high cholesterol diet (HCD) group (n=5) and was fed with the 12% cholesterol diet. The experimental diets were administered for 6 consecutive weeks.

Biochemical study

Blood specimens were collected at completed 48 hours, 7 days and 6 weeks and analysed for renal function test and lipid profile (Siemen Xpand Plus, USA).

Histological study

At the completed 6 weeks, the rats were euthanized and both kidneys were harvested and fixed in 10% neutral buffered formalin for histological examination. The kidneys were processed using automated tissue processor (Leica TP 1020). The tissues were embedded into paraffin blocks (Leica EG1160) and sectioned at 3 μm thickness and stained with hematoxylin and eosin (H&E) stain and Masson trichrome stain.

Statistical analysis

Statistical analysis was performed using Student's t-test available in the statistical programme SPSS version 20.0 to compare renal function test and lipid profile parameters of the study groups. A value of $p<0.05$ was considered to be significant. The histological sections were analysed by two pathologists randomly.

Results

The lipid profile

The lipid profile results for the control group and HCD group is as shown in Table 1 The total cholesterol levels exhibited an increasing trend throughout the experiment (from 48 hours to 7 days to 6 weeks).

The mean serum total cholesterol level in the HCD group were significantly higher ($p= 0.021$) as compared to the control group at completed 7 days.

Also the mean serum total cholesterol was significantly higher ($p= 0.04$) in the HCD group in comparison to the control group at 6 weeks. The mean serum HDL-c level and triglyceride levels in the HCD group were however lower ($p = 0.003$ and 0.045 respectively) as compared to the control at 6 weeks.

Table 1. Lipid profile in the control and HCD groups of rats

Lipid Profile parameter (mmol/L)	48-hours		7 Days		6 week	
	Control	HCD	Control	HCD	Control	HCD
Cholesterol	1.58±0.79	2.30±0.40	1.90±0.25	2.84±0.68*	2.06±0.38	7.38±2.93*
HDL-c	1.90±0.16	1.88±0.21	1.82±0.24	2.45±0.65	0.47±0.08	0.23±0.10*
Triglyceride	0.42±0.10	0.50±0.20	0.36±0.08	0.40±0.10	0.82±0.22	0.53±0.15*

Values are given as means ± sd. Significant differences were analyzed using Student's t-test, and indicated as *$p<0.05$ when comparing control with HCD rats.

The renal function test

Table 2 shows the renal profile results of the two experimental groups. The mean serum creatinine level increased significantly after 48 hours ($p = 0.004$) and 7 days ($p = 0.028$) in the HCD as compared to the control group.

None of the other renal profile parameters (urea, sodium, potassium, chloride and uric acid) showed significant difference at 48 hours, 7 days and 6 weeks.

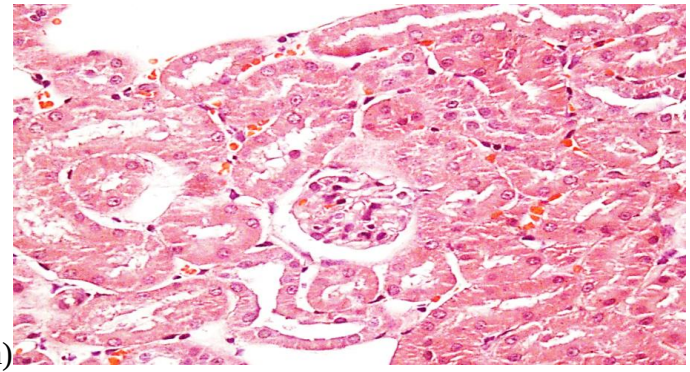
Table 2. Renal profile in the control and HFD groups of rats

Renal Profile parameter	48-hours		7 Days		6 week	
	Control	HCD	Control	HCD	Control	HCD
Creatinine (µmol/L)	25.00±4.80	37.25±3.59*	19.00±5.40	33.00±8.89*	35.60±7.96	38.75±4.28
Urea (mmol/L)	4.66±0.67	4.56±1.05	7.78±0.94	9.24±4.07	7.40±0.82	9.76±4.20
Sodium (mmol/L)	138.20±1.30	137.40±0.55	137.20±1.30	136.80±2.17	139.00±1.00	139.20±2.05
Potassium (mmol/L)	5.06±0.60	5.38±0.29	5.96±0.83	7.18±1.86	4.68±0.41	4.40±0.48
Chloride (mmol/L)	101.80±1.64	100.40±0.55	100.60±2.07	100.20±0.84	101.00±1.87	99.80±1.48
Uric acid (mmol/L)	0.13±0.03	0.11±0.05	0.17±0.02	0.16±0.04	0.12±0.04	0.13±0.03

Values are given as means ± SD. Significant differences were analysed using student t-test, and indicated as *p<0.05 when comparing control with HCD rats.

The renal histology

In the control group, the histopathological examination of the kidney sections at 6 weeks revealed normal cortical and medullary pattern with well formed glomeruli, normal tubules and tubulointersitium [Fig. 1(a)]. In the sections stained with the Masson's trichrome, the kidneys showed no areas of increased fibrosis [Fig. 1(b)].The sections of the kidneys from the HCD group showed segmental mesangial hypercellularity with segmental mesangial matrix expansion of most glomeruli in both kidneys. No other renal histopathological changes were seen in this group [Fig. 2(a)]. As for the Masson trichrome stained sections, the kidney showed similar histological picture to that of the control group, with no evidence of increased amount of periglomerular or peritubular fibrous tissue [Fig. 2(b)].

1(a) 

1(b)

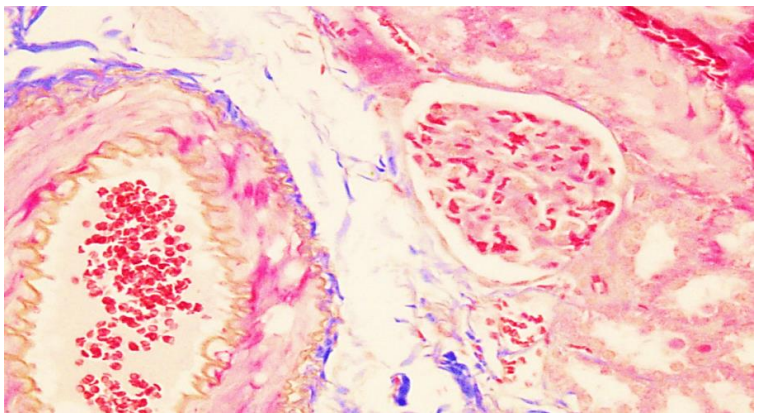
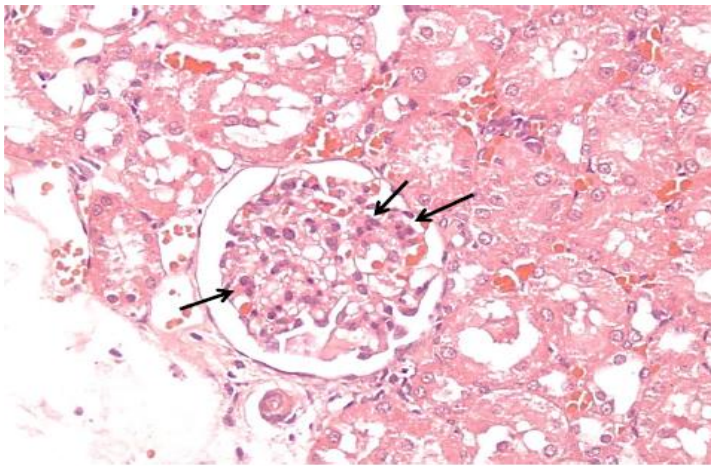


Fig. 1 Representative (a) H&E-stained section (x40 objective) and (b) Masson trichrome-stained section (x40 objective) of a kidney from a control animal without visible pathological changes.

2(a)



2(b)

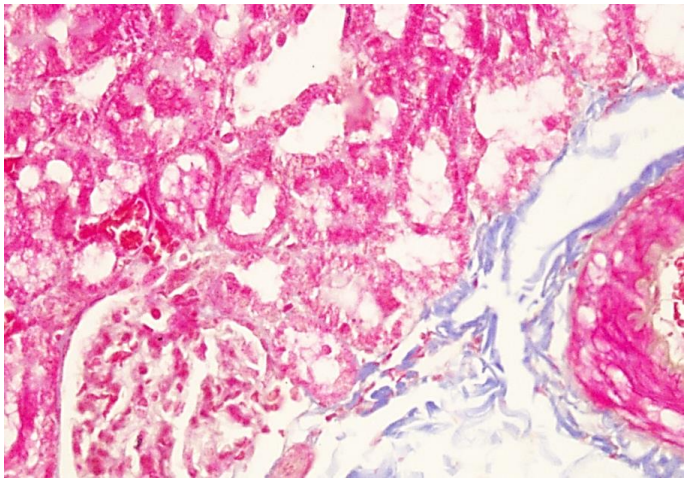


Fig. 2 Representative (a) H&E-stained section (x40 objective) showing segmental mesangial proliferation with mesangial matrix expansion of the glomeruli (arrow) and (b) Masson trichrome-stained section (x40 objective) exhibiting of no area of increased amount of periglomerular or peritubular fibrous tissue formation.

Discussion

In our study the consumption of 12% cholesterol diet by the animal resulted in dyslipidaemia as evident by the significantly increased serum total cholesterol levels at completed 7 days and 6 weeks. A similar results were obtained by Mohd Adzim Khalili et al., 2009 (Mohd Adzim Khalili et al., 2009). Additionally there was significant reduction in the serum HDL-c levels at 6 weeks reported by Matos et al. (2005). Alsaif et al. (2007) however reported a contradicting result where 1% high cholesterol diet-fed hamsters showed a significant elevation of plasma HDL-c levels after 13 weeks.

It is however interesting to note that the mean triglyceride level at 6 weeks in the HCD was significantly lower than the control. No similar result has been reported previously. Earlier studies reported either an increase in serum triglyceride level with high cholesterol diet administration (Abdel-Hafez, Othman, & Seleim, 2011), or lack of significant differences in the triglyceride with high cholesterol diet supplementation (Matos et al., 2005). In this study, we did not report the LDL-c level, because the Friedewald formula used for calculation of LDL-c is not advisable to be utilised in hypercholesterolemic rats as it will overestimate the LDL-c (Sanchez-Muniz & Bastida, 2008).

The serum creatinine levels were also elevated in HCD group compared to control group at 48 hours and 7 days. There are very limited available published studies related to the acute nephrotoxic effects of high cholesterol diet. Most studies were conducted to examine the sub-acute and chronic effects of high cholesterol diet induced kidney injury. El-wakf et al., (2014) reported increased levels of serum creatinine, blood urea, uric acid and serum potassium with reduction of serum sodium with 2% cholesterol diet in combination with 30% fat diet for 12 weeks (El-wakf et al., 2014). In another study by Joles et al. (2000) revealed that high cholesterol diet (2%) did not have any effect on the serum creatinine level although the study was carried out on uninephrectomy rats for 13 weeks (Joles et al., 2000).

In addition to the significant increase of serum creatinine, the 12% cholesterol diet also induced histological changes characterised by segmental mesangial hypercellularity with mesangial matrix expansion of the renal glomeruli in the kidney tissues at 6 weeks. We however did not examine the kidney tissues at 48 hours and 7 days. Mesangial cells are target cells of hyperlipidaemia since they have the ability to bind lipoproteins through receptors expressed and this can in turn lead to lipid accumulation and subsequently dysfunctional glomeruli (Bruneval et al., 2002). They also have a role in inducing glomerular injury due to their major roles in the production of extracellular matrix. Our renal histological findings of the HCD group conformed to that reported by Ghada et al. (2014) who described similar features with 4% cholesterol administration for 8 weeks, in addition to other histopathological changes not observed in ours. Al-Rejaie

et al. (2012) has reported in his study where on gender difference in renal injury induced by high cholesterol diet for 6 weeks causes hypercellular glomeruli with mesangial proliferation.

High cholesterol diet has been shown to cause kidney damage via different mechanisms and they include two pertinent processes inflammation and oxidative stress (He et al., 2015). Fat accumulation in the kidney is believed to contribute to the pathogenesis (Camer et al., 2016) as it is associated with macrophage infiltration which activates the release of pro-inflammatory cytokines including tumour necrosis factor alpha resulting in kidney damage (Camer et al., 2016). In addition, ectopic fat accumulation (the accumulation of lipid in non-adipose tissue) leads to production of toxic metabolites (Unger & Scherer, 2010) which can cause dysfunction of the mitochondria, stress of endoplasmic reticulum, apoptosis, and finally causing dysfunction and injury of the kidney (Hall et al., 2014).

Conclusion

The 12% cholesterol diet induced dyslipidaemia in the animal model and had resulted in kidney injury as evident by the increased in the mean serum creatinine level. Histological examination of the kidney tissues at 6 weeks revealed changes confined to the mesangial cells of the glomeruli of the kidney.

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