Association of obesity with chronic kidney disease in elderly patients with nonalcoholic fatty liver disease

Kexue Luo[1] ⓘ, Jie Bian[2] ⓘ, Qinxian Wang[1] ⓘ, Jiesheng Wang[1] ⓘ, Fuxing Chen[1] ⓘ, Hongchun Li[1] ⓘ, Dong Jin[1] ⓘ

[1]Tongde Hospital of Zhejiang Province, Hangzhou, Zhejiang, China
[2]Sir Run Run Shaw Hospital, International Medical Center, Zhejiang University, Zhejiang, China

ABSTRACT

Background/Aims: This study investigated an association between obesity and impaired renal functions in elderly patients with nonalcoholic fatty liver disease (NAFLD) and evaluated the risk factors for chronic kidney disease (CKD) in these patients.
Materials and Methods: A cross-sectional study was performed involving 515 elderly patients (≥ 60 years old) with NAFLD. Demographics, body mass index (BMI), medical history, and laboratory parameters were compared for groups stratified by obesity (≥ 28 kg/m2) or CKD. An association between obesity and CKD was analyzed, and a multivariate logistic regression analysis was conducted for risk factors associated with CKD.
Results: In the overall population, 28.7% were obese and 54.8% had CKD; there were more women (58.8%) than men. The prevalence of hypertension and diabetes was similar between the obese and nonobese groups and between the CKD and non-CKD groups. Obese patients had significantly higher levels of serum uric acid and estimated glomerular filtration rates when compared with the nonobese group. When compared with those without CKD, patients with CKD were significantly older in addition to having higher BMI and serum uric acid levels. The multivariate logistic regression analysis indicated that CKD was positively associated with age, BMI, and serum uric acid levels.
Conclusion: Elderly obese patients with NAFLD are at a higher risk of CKD. NAFLD patients with advanced age, greater BMI, or higher serum uric acid levels are more prone to developing CKD. The renal function of NAFLD patients should be closely monitored.
Keywords: Elderly patients, nonalcoholic fatty liver disease, obesity, chronic kidney disease, uric acid

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) refers to the accumulation of fat in the liver that is not due to heavy alcohol consumption (1). NAFLD is the most common cause of chronic liver disease in developed countries, with a prevalence from 7% to 33% (2). Without appropriate diagnosis and treatments, NAFLD can progress to fibrosis, cirrhosis, or even hepatocellular carcinoma (3).

The common risk factors of NAFLD include obesity and diabetes (4). Obesity can induce alterations in adipose tissue cellular composition and functions. This has been termed as adipose tissue remodeling (5). Adipose tissue remodeling can promote adipocyte dysfunction, abnormal cytokine secretions, and persistent low-grade inflammatory responses. Obesity can also induce ectopic fat deposition in non-adipose tissues. The microenvironment of ectopic fat can disrupt the normal balance between proinflammatory and antiinflammatory cytokines, which can contribute toward the pathogenesis of NAFLD (6). It has been demonstrated that the majority of mor-

bidly obese patients had NAFLD, and one-third of them had steatohepatitis (7).

In addition to NAFLD, decreased renal functions are also associated with obesity and diabetes: obesity is an established risk factor for chronic kidney disease (CKD) (8). Patients who are overweight or obese are more likely to have decreased glomerular filtration rate (GFR), a major indication for renal function (9); further, diabetes can cause renal failure (10).

Since diabetes and obesity are risk factors of both NAFLD and CKD, an association has been shown between CKD and NAFLD (11,12). Pathophysiological mechanisms that lead to the development of CKD in patients with NAFLD include insulin resistance, inflammation, oxidative stress, and fibrogenesis (13-15).

Age is also associated with declined renal functions (16). However, it has not been determined whether obesity can specifically exacerbate the risk of kidney disease in

elderly patients with NAFLD or not. If so, the evaluations of renal functions should be performed more frequently in such patients in order to promptly treat renal damage.

This study investigated an association between obesity and CKD in elderly patients with NAFLD and evaluated the risk factors for CKD in these patients.

MATERIALS AND METHODS
This was a cross-sectional study conducted between April 2015 and December 2016 at the Tongde Hospital of Zhejiang Province, China. The hospital's ethics committee approved the study protocol. All the study participants provided signed informed consent.

Participants
The study population consisted of patients aged ≥ 60 years with NAFLD who visited the medical clinic or were hospitalized in an urban hospital in China. The diagnosis of NAFLD was based on the global guidelines of the World Gastroenterology Organization (17). Since liver biopsy and histopathological examination are not routine procedures in the clinic, ultrasound examination was used to identify patients with diffuse fatty liver disease. This disease was diagnosed in patients with at least two of the following three abnormal findings in the ultrasound examination: diffusely increased liver near field ultrasound echo ("bright liver"), i.e., liver echo greater than that of the kidney; vascular blurring; and gradual attenuation of far field ultrasound echo.

Patients with any of the following were excluded from this study: chronic viral hepatitis; hepatolenticular degeneration (Wilson's disease); alpha antitrypsin deficiency; primary biliary cirrhosis; hemochromatosis; alcoholism; drug use; schistosomiasis; autoimmune liver disease; hypothyroidism or Cushing's syndrome; malignant tumors; acute renal disease (abnormal renal function within ≤3 months); or kidney transplant patients. In addition, patients who recently used medications that could cause liver steatosis, such as amiodarone, methotrexate, or glucocorticoids, were excluded.

Study protocol
Patients' baseline characteristics (age, gender, weight, and height) and medical history (including hypertension and diabetes) were documented. Blood was drawn during the early morning after overnight fasting. The following laboratory parameters were measured with a Hitachi 7600-110 automatic biochemical analyzer (Hitachi, Japan) in accordance with the standard hospital laboratory protocol: serum alanine aminotransferase (ALT) and creatinine (SCr); fasting blood glucose; uric acid; triglyceride; and total, high-density, and low-density lipoprotein cholesterol values. Liver ultrasound was performed by an experienced ultrasound physician by using a Philips HD11XE instrument (Philips, the Netherlands).

Measurement outcomes
Obity was considered when the body mass index (BMI) ≥ 28 kg/m^2 (18). Diabetes was based on the following: clinical symptoms (polydipsia, polyuria) with blood glucose ≥ 200 mg/dL at any time; fasting blood glucose ≥ 126 mg/dL; 2-h blood glucose ≥ 200 mg/dL during a glucose tolerance test; or currently taking hypoglycemic agents. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic pressure ≥ 90 mmHg, or currently on antihypertensive agents. The estimated GFR (eGFR) was calculated based on the following CKD epidemiology collaboration (CKD-EPI) equation (19):

$$eGFR = 141 \times min(SCr/\kappa, 1)^\alpha \times max(SCr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ (if female)}$$

where SCr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, age is in years, min indicates the minimum of SCr/κ or 1, and max is the maximum of SCr/κ or 1.

Chronic kidney disease was diagnosed if a patient had any of the following for 3 months: abnormal urine sedimentation; renal tubular diseases; abnormal histological study; abnormal structures in imaging study; history of kidney transplantation; eGFR < 60 mL/(min: 1.73 m^2); albuminuria (urinary albumin excretion rate ≥ 30 mg/24 h; urinary albumin/creatinine ratio ≥ 30 mg/g or ≥ 3 mg/mmol) (20).

Statistical analysis
Data with normal distributions are presented as mean ± standard deviation. Data without normal distributions were analyzed after logarithmic conversion. Continuous variables were compared using Student's t-test or analysis of variance. Categorical variables were compared by chi-squared analysis. Multivariate logistic regression was performed after first examining the bivariate association by correlation analyzes. $p < 0.05$ was considered to be statistically significant. Statistical software Statistical Package for Social Sciences (SPSS version 19.0, IBM Corp.; Armonk, NY, USA) was used for all the statistical analyzes.

RESULTS
Altogether, 515 elderly patients with NAFLD were enrolled in the current study (age: 68.5±6.9 years; Table 1). Out of these patients, 28.7% (148/515) were obese, 54.8% (282/515) had CKD, and there were more wom-

en (303/515, 58.8%) than men (212/515, 41.2%) in the study population. The overall study population included 268 patients (52.0%) with hypertension and 96 patients with diabetes (18.6%).

Table 1. Demographic and laboratory results of the obese and non-obese groups

	Obese	Non-obese	p
Subjects, n	148	367	-
Age, year	69.6±7.2	68.0±6.7	0.09
Female, n (%)	96 (64.9%)	207 (56.4%)	0.05
BMI, kg/m2	29.6±1.7	25.3±1.6	<0.01
Hypertension, n (%)	77 (52.0%)	191 (52.0%)	0.54
Diabetes, n (%)	30 (20.3%)	66 (18.0%)	0.31
CKD, n (%)	94 (63.5%)	188 (51.2%)*	<0.01
Serum ALT, IU/L	21.4±16.7	20.6±12.7	0.55
Serum Cr, μmol/L	97.1±13.6	97.0±14.5	0.76
Uric acid, μmol/L	376.7±83.5	358.3±85.0	0.05
Fasting blood glucose, mg/dL	111.3±28.6	111.6±31.1	0.91
Total cholesterol, mg/dL	191.1±39.6	188.9±37.2	0.54
Triglyceride, mg/dL	170.8±75.3	177.1±106	0.45
High-density lipoprotein, mg/dL	49.8±10.0	50.5±12.8	0.54
Low-density lipoprotein, mg/dL	127.2±39.9	122.6±35.8	0.21
eGFR, mL/min/1.73 m^2	58.0±10.3	60.3±10.0 *	0.01

*A significant difference between the obese and non-obese groups; p< 0.05
ALT: alanine aminotransferase; Cr: creatinine

Table 2. Demographic and laboratory results of the CKD and non-CKD groups

	CKD	Non-CKD	p
Subjects	282	233	—
Age, year	70.9±7.2	65.5±5.1*	<0.01
Female, n (%)	135 (47.9%)	107 (46.0%)	0.72
BMI, kg/m^2	27.3±2.6	25.6±2.1*	<0.01
Hypertension, n (%)	151 (53.5%)	117 (50.2%)	0.48
Diabetes, n (%)	52 (18.4%)	44 (18.9%)	0.91
Serum ALT, IU/L	21.6±16.0	19.7±11.0	0.12
Uric acid, μmol/L	379.3±93.3	342.9±72.1 *	<0.01
Fasting blood glucose, mg/dL	111.5±30.6	111.6±30	0.96
Total cholesterol, mg/dL	193.3±39	186.9±36.1	0.11
Triglyceride, mg/dL	179.9±97.7	169.7±98.5	0.24
High-density lipoprotein, mg/dL	50.5±13.3	50.1±10.4	0.72
Low-density lipoprotein, mg/dL	126.4±38.1	120.9±35.6	0.09

*A significant difference between the CKD and non-CKD groups
ALT: alanine aminotransferase

When compared with the nonobese group, obese patients had a significantly higher percentage of women and a significantly higher rate of CKD (Table 1). Uric acid levels were significantly higher in the obese than the nonobese group, and eGFR was significantly lower. The groups were comparable with regard to age and rates of hypertension and diabetes.

When comparing patients with and without CKD, the former had significantly older and with significantly higher BMI and serum uric acid levels (Table 2).

The multivariate logistic analysis showed that age, BMI, and serum uric acid levels were significantly associated with CKD in patients with NAFLD (Table 3).

DISCUSSION

Impaired renal function in elderly patients can be easily missed, since more attention is usually paid to cardiovascular functions. However, this study shows that elderly obese patients with NAFLD are more prone than nonobese patients in developing CKD. In particular, our results indicate that age, obesity, and serum uric acid levels were positively associated with CKD in patients with NAFLD. NAFLD patients with advanced age, greater BMI, or high serum uric acid level were more likely to have renal insufficiency. Therefore, NAFLD patients with these characteristics require more frequent evaluation of their renal functions.

According to the multiple-hit hypothesis, many factors contribute toward hepatic steatosis and inflammation, such as lipid deposition, insulin resistance, hormone imbalance, nutritional factors, oxidative stress and cytokine activations, bacterial overgrowth, and genetic and epigenetic factors (21). Many of these factors could also potentially cause renal damage.

Age is an independent risk factor for CKD (16). Elderly patients commonly have atherosclerosis, chronic inflammation, and increased oxidative stress (22). All of these can contribute to a lower GFR value and decreased renal functions.

In obese patients, lipid accumulation can cause nephrotoxicity (23). Macrophages and glomerular mesangial cells can oxidize lipoprotein. After the uptake of oxidized lipoprotein, macrophages can transform into foam cells, which can release a variety of inflammatory cytokines and promote mesangial matrix production. These finally result in glomerulosclerosis and decreased GFR and renal

Luo et al. Obesity, chronic kidney disease, elderly NAFLD

Turk J Gastroenterol 2019; 30(7): 611-5

functions (24). In patients with NAFLD, fat accumulation in the liver cells can accelerate energy metabolism and increase oxidative stress, which can activate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and c-Jun N-terminal kinase (JNK) signaling pathways. This can further disrupt the systemic inflammatory immune system and exaggerate oxidative stress, finally leading to kidney damage (14,25). Studies have suggested that in the presence of NAFLD, elevated fetuin-A levels might impair renal function by renal sinus fat-induced proinflammatory signaling in glomerular cells (26).

Studies have shown that obesity itself can cause kidney injury (27, 28). Obese patients commonly have elevated GFR, which can increase sympathetic tone and cardiac output, as well as cause hyperinsulinemia (29). These changes can result in renal podocyte hypertrophy and intracellular lipid deposition, which eventually progresses into segmental and focal glomerulosclerosis and renal interstitial fibrosis. In addition, obesity can disrupt the balance of the renin-angiotensin-aldosterone system and increase the release of angiotensin and aldosterone (30). Both angiotensin and aldosterone can impair liver and kidney functions. Obese patients have higher levels of adiponectin and lower levels of leptin, which also contribute to the development of kidney damage (31).

Recent studies have suggested that a steatotic and inflamed liver can cause kidney injury by secreting proinflammatory, profibrogenic, and antifibrinolytic cytokines such as fetuin-A, fibroblast growth factor (FGF)-21, tumor necrosis factor (TNF)-a, transforming growth factor (TGF)-b, and plasminogen activator inhibitor-1. In addition, oxidized low-density lipoproteins and triglyceride-rich lipoproteins, which can have increased levels due to the overproduction of very-low-density lipoprotein and the promotion of atherogenic dyslipidemia from the fatty liver, could induce mesangial cell proliferation and glomerular injury, ultimately resulting in renal injury (32).

An earlier study has reported an association between CKD and high uric acid levels (33). Our current study confirmed this and showed that the uric acid level was significantly associated with CKD in patients with NAFLD, i.e., patients with CKD had higher uric acid levels. Whether high uric acid causes CKD or is a result of CKD requires further investigations.

Recent studies have reported that microalbuminuria may be found in patients with NAFLD. It is commonly observed that patients with NAFLD and microalbuminuria have higher insulin resistance, which may lead to microalbuminuria through endothelial dysfunction, glomerular hyperfiltration, increased vascular permeability, and altered microstructure of the renal corpuscles (34, 35). Both microalbuminuria and high insulin resistance can lead to kidney damage. In the current study, we did not measure insulin resistance or microalbuminuria. Future studies may investigate whether NAFLD patients with microalbuminuria or insulin resistance have a higher risk for CKD relative to other NAFLD subgroups.

This study is limited as a single-center study with a cross-sectional design, which could not support a causal association between NAFLD and CKD, as well as that between uric acid level and CKD. Patients' renal functions were estimated with the CKD-EPI equation, which is widely used in clinics. Further, we used a lower cutoff value for obesity (BMI ≥ 28 kg/m^2) in the current study, which may differ from the defined values in other countries. We did not observe statistically significant differences in hypertension or diabetes between the obese and nonobese groups. This may be because we only studied elderly NAFLD patients. Old age and NAFLD are risk factors for hypertension and diabetes, and the ratio of women-to-men was higher in the obese group. Smoking is an established risk factor for CKD; unfortunately, we did not ask for patient's smoking status during the data collection process. Any of these factors might have confounded the study results. In addition, we used ultrasound examination, but not liver biopsy or histological examination, to identify patients with fatty liver disease. Ultrasound examination has its limitations and can vary among examiners. Future large cohort studies with longer follow-up periods and histologically confirmed fatty liver disease are needed to verify the association between NAFLD and CKD that was determined in our study.

In conclusion, this study found that elderly obese patients with NAFLD were more likely to have CKD as compared to those who were not obese. The multivariate logistic regression analysis showed that CKD was also significantly associated with advanced age, greater BMI, and higher serum uric acid level. Our results indicate that an early evaluation of renal functions should be performed in NAFLD patients with these characteristics in order to initiate prompt treatments and prevent chronic renal disease.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - K.L.; Design - J.S.W.; Supervision - R.W.; Resources - F.C.; Materials - H.L.; Data Collection and/or Processing - J.B.; Analysis and/or Interpretation - K.L.; Literature Search - D.J.; Writing Manuscript - K.L.; Critical Review - J.B.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. World J Hepatol 2017; 9: 715-32. **[CrossRef]**

2. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. Dig Dis 2010; 28: 155-61. **[CrossRef]**

3. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012; 142: 1592-609. **[CrossRef]**

4. Streba LA, Vere CC, Rogoveanu I, Streba CT. Nonalcoholic fatty liver disease, metabolic risk factors, and hepatocellular carcinoma: an open question. World J Gastroenterol 2015; 21: 4103-10. **[CrossRef]**

5. Itoh M, Suganami T, Hachiya R, Ogawa Y. Adipose tissue remodeling as homeostatic inflammation. Int J Inflam 2011; 2011: 720926. **[CrossRef]**

6. Yilmaz Y, Younossi ZM. Obesity-associated nonalcoholic fatty liver disease. Clin Liver Dis 2014; 18: 19-31. **[CrossRef]**

7. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol 2006; 45: 600-6. **[CrossRef]**

8. Wickman C, Kramer H. Obesity and kidney disease: potential mechanisms. Semin Nephrol 2013; 33: 14-22. **[CrossRef]**

9. Vinhas J, Gardete-Correia L, Boavida JM, et al. Prevalence of chronic kidney disease and associated risk factors, and risk of end-stage renal disease: data from the PREVADIAB study. Nephron Clin Pract 2011; 119: c35-40. **[CrossRef]**

10. Koye DN, Shaw JE, Reid CM, Atkins RC, Reutens AT, Magliano DJ. Incidence of chronic kidney disease among people with diabetes: a systematic review of observational studies. Diabet Med 2017; 34: 887-901. **[CrossRef]**

11. Targher G, Byrne CD. Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. Nat Rev Nephrol 2017; 13: 297-310. **[CrossRef]**

12. Yasui K, Sumida Y, Mori Y, et al. Nonalcoholic steatohepatitis and increased risk of chronic kidney disease. Metabolism 2011; 60: 735-9. **[CrossRef]**

13. Chen J, Muntner P, Hamm LL, et al. Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. J Am Soc Nephrol 2003; 14: 469-77. **[CrossRef]**

14. Ix JH, Sharma K. Mechanisms linking obesity, chronic kidney disease, and fatty liver disease: the roles of fetuin-A, adiponectin, and AMPK. J Am Soc Nephrol 2010; 21: 406-12. **[CrossRef]**

15. Marcuccilli M, Chonchol M. NAFLD and Chronic Kidney Disease. Int J Mol Sci 2016; 17: 562. **[CrossRef]**

16. Kazancioglu R. Risk factors for chronic kidney disease: an update. Kidney Int Suppl (2011) 2013; 3: 368-71. **[CrossRef]**

17. Review T, LaBrecque DR, Abbas Z, et al. World Gastroenterology Organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Clin Gastroenterol 2014; 48: 467-73.

18. He W, Li Q, Yang M, et al. Lower BMI cutoffs to define overweight and obesity in China. Obesity (Silver Spring) 2015; 23: 684-91. **[CrossRef]**

19. Pei X, Bao L, Xu Z, et al. Diagnostic value of cystatin C and glomerular filtration rate formulae in Chinese nonelderly and elderly populations. J Nephrol 2013; 26: 476-84. **[CrossRef]**

20. Stevens PE, Levin A, Kidney Disease: Improving Global Outcomes Chronic Kidney Disease Guideline Development Work Group M. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. Annals of internal medicine 2013; 158: 825-30. **[CrossRef]**

21. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism 2016; 65: 1038-48. **[CrossRef]**

22. Schleicher E, Friess U. Oxidative stress, AGE, and atherosclerosis. Kidney international Supplement 2007: S17-26. **[CrossRef]**

23. Ruan XZ, Varghese Z, Moorhead JF. An update on the lipid nephrotoxicity hypothesis. Nat Rev Nephrol 2009; 5: 713-21. **[CrossRef]**

24. Kzhyshkowska J, Neyen C, Gordon S. Role of macrophage scavenger receptors in atherosclerosis. Immunobiology 2012; 217: 492-502. **[CrossRef]**

25. Massy ZA, Stenvinkel P, Drueke TB. The role of oxidative stress in chronic kidney disease. Seminars in dialysis 2009; 22: 405-8. **[CrossRef]**

26. Wagner R, Machann J, Guthoff M, et al. The protective effect of human renal sinus fat on glomerular cells is reversed by the hepatokine fetuin-A. Scientific reports 2017; 7: 2261. **[CrossRef]**

27. Kalaitzidis RG, Siamopoulos KC. The role of obesity in kidney disease: recent findings and potential mechanisms. International urology and nephrology 2011; 43: 771-84. **[CrossRef]**

28. Mallamaci F, Tripepi G. Obesity and CKD progression: hard facts on fat CKD patients. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2013; 28 Suppl 4: iv105-8. **[CrossRef]**

29. Knight SF, Imig JD. Obesity, insulin resistance, and renal function. Microcirculation 2007; 14: 349-62. **[CrossRef]**

30. Noguchi R, Yoshiji H, Ikenaka Y, et al. Selective aldosterone blocker ameliorates the progression of non-alcoholic steatohepatitis in rats. International journal of molecular medicine 2010; 26: 407-13.

31. Tilg H. The role of cytokines in non-alcoholic fatty liver disease. Dig Dis 2010; 28: 179-185. **[CrossRef]**

32. Musso G, Gambino R, Tabibian JH, et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med 2014; 11: e1001680. **[CrossRef]**

33. Moe OW. Posing the question again: does chronic uric acid nephropathy exist? J Am Soc Nephrol 2010; 21: 395-7. **[CrossRef]**

34. Parvanova AI, Trevisan R, Iliev IP, et al. Insulin resistance and microalbuminuria: a cross-sectional, case-control study of 158 patients with type 2 diabetes and different degrees of urinary albumin excretion. Diabetes 2006; 55: 1456-62. **[CrossRef]**

35. Yilmaz Y, Alahdab YO, Yonal O, et al. Microalbuminuria in non-diabetic patients with nonalcoholic fatty liver disease: association with liver fibrosis. Metabolism 2010; 59: 1327-30. **[CrossRef]**