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### ORIGINAL RESEARCH

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## Investigating the causal relationship between human blood/ urine metabolites and periodontal disease using two-sample Mendelian randomization

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### Abstract

**Background and Aims:** The aim is to investigate the cause-and-effect connection between metabolites found in blood/urine and the likelihood of developing periodontal disease (PD) through the utilization of a two-sample Mendelian randomization (MR) method.

**Methods:** Using an inverse variance weighted (IVW) method and two additional twosample MR models, we examined the relationship between blood/urine metabolites and PD by analyzing data from a comprehensive metabolome-based genome-wide association study and the Genome-Wide Association Studies (GWAS) of PD. To assess the consistency and dependability of the findings, diversity, cross-effects, and sensitivity analyses were conducted.

**Results:** Out of the 35 metabolites found in blood and urine, a total of eight metabolites (C-reactive protein, Potassium in urine, Urea, Cystatin C, Non-albumin protein, Creatinine, estimated Glomerular Filtration Rate, and Phosphate) displayed a possible causal connection with the risk of dental caries/PD using the inverse variance weighted (IVW) method (p < 0.05). This includes five metabolites in the blood and three in the urine. No metabolites were statistically significant in IVW MR models ( $p < 3.68 \times 10^{-4}$ ). Even after conducting sensitivity analysis with the leave-one-out method and removing the confounding instrumental variables, the impact of these factors on dental caries/PD remained significant.

**Conclusion:** Based on the available evidence, it is not possible to establish a significant causal link between the 35 blood metabolites and the likelihood of developing dental caries and PD.

### KEYWORDS

dental caries, Mendelian randomization, metabolite, periodontal disease

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### 1 | INTRODUCTION

Dental hygiene plays a crucial role in global physical well-being. Dental caries and periodontal diseases, like gingivitis and periodontitis, are the prevalent ailments that impact oral well-being. According to the 2016 study on the Global Burden of Disease, Injury, and Risk Factors, dental caries in permanent teeth and periodontitis were identified as the primary and eleventh most widespread sources of illness globally in 2016.<sup>1</sup> It is crucial to identify the cause of these illnesses as they impose substantial health and financial consequences, with dental disease worldwide costing over \$540 billion in 2015.<sup>2,3</sup> Dental caries is a disease process that can lead to irreversible damage to dental tissue.<sup>4,5</sup> The Decayed, Missing, and Filled teeth index (DMF index), which includes decayed, missing, and filled teeth for both primary and permanent dentition, is a widely employed approach for measuring the prevalence of dental caries.<sup>6</sup> Meanwhile, Periodontal disease, a condition characterized by inflammation in the supporting tissues of the teeth caused by microorganisms, impacts around half of the adult population, with 10% experiencing the severe form of the disease.<sup>7</sup> According to recent studies on the global burden of disease (1990-2017), it has been estimated that around 796 million individuals experience severe periodontitis, with a reported age-standardized prevalence of 9.8%.<sup>8</sup> Prior research has indicated that numerous changeable risk factors are linked to dental cavities and gum disease, including inadequate eating patterns (for instance, regular intake of processed sugars), substandard dental care, tobacco use, and alcohol consumption.<sup>9-11</sup> Nevertheless, there are still unidentified modifiable factors linked to dental caries and periodontitis.

To routinely assess and track chronic illnesses, measuring biomarkers in serum and urine at regular intervals is a standard practice.<sup>12</sup> Knowing the genetic predisposition to particular biomarker conditions and the variables that complicate them could potentially impact the management of diseases. While lipids,<sup>13,14</sup> glycemic traits,<sup>15,16</sup> and measures of renal function<sup>17,18</sup> have been thoroughly investigated, the genetic aspects of certain biomarkers have also been extensively examined. Large population-scale datasets have not been used to investigate the genetic foundation of most biomarkers.<sup>19</sup> The most extensive Genome-Wide Association Studies (GWAS) up to now has mapped the genetic blueprint of human blood metabolites,<sup>19</sup> offering a significant benchmark for the genetic foundation of blood and urine metabolomics. We explored the cause-and-effect connection between these blood metabolites and the susceptibility to periodontal disease and the formation of cavities using this approach. Although it has not been reported yet, gaining more understanding of the pathogenesis of periodontal disease and caries can offer fresh perspectives on the clinical treatment of patients with these conditions.

Mendelian randomization (MR) offers an alternative approach to address the issue of observational bias.<sup>20,21</sup> In MR, genetic information is utilized as an arbitrary origin of exposed diversity, ensuring that the origin of diversity remains unaffected by confounding factors.<sup>22,23</sup> By harnessing the inherent variability in an individual's

genetic composition, similar to the approach of a randomized controlled trial, this method utilizes genetic variation in instrumental variable analysis to deduce the impact of a modifiable exposure on an outcome. Therefore, MR offers a dependable comprehension of the impacts of alterable exposures on characteristics of concern in contrast to conventional observational studies that are vulnerable to confounding or reverse causality.<sup>23</sup>

Hence, in this study, a two-sample MR analysis was conducted to explore the causal association between 35 blood/urine metabolites and the progression of dental caries and periodontal disease. The analysis was performed from a molecular mechanism viewpoint, utilizing the aforementioned extensive GWAS data as the exposure data and an additional vast GWAS data on periodontal disease and dental caries as the outcome data. Additionally, this research possesses a specific foundation in theory and holds significance in clinical application. The findings can serve as a guide for the advancement of tools used in predicting and treating dental caries and periodontal disease.

### 2 | MATERIALS AND METHODS

### 2.1 | Design of the study

To explore the potential causal connection between exposure and the outcome of interest, genetic variations closely linked to exposure will serve as instrumental variables in MR analyses.<sup>24</sup> To enhance inferences about the potential causal impact on outcomes, MR utilizes genetic variants associated with exposure. The method utilizes Mendel's principles of separation and autonomous categorization, where genetic variations are assigned autonomously without considering environmental and other genetic factors (except for variations nearby due to linkage disequilibrium [LD]).<sup>23,25</sup> For each IV to be considered valid, three key conditions must be met: (1) a strong connection between the instrument and the exposure; (2) the instrument affecting the outcome solely through the exposure; and (3) genetic variation being unrelated to factors that may confound the association between exposure and outcome.<sup>26</sup> The findings of this research are presented following the guidelines provided by STROBE-MR and the Mendelian Randomization Survey Guidelines.<sup>27</sup> The study procedure and specifics were not previously registered.

### 2.2 Data sources

The exposure data for this study was obtained from the largest GWAS data published in Nature Genetics in 2021, conducted by Armstrong et al.<sup>19</sup> The meta-analysis includes 363,228 Europeans who underwent rigorous quality control. It encompasses a comprehensive set of 2.1 million SNP loci and 35 blood and urine metabolites for conducting genome-wide association analysis. These metabolites can be divided into several metabolite categories: lipids, glycemic traits, and vitamins, energy

products, heterologous biological metabolites. The database website 10.35092/yhjc.12355382 provides public access to summary data for all association analyses. Table 1 contains comprehensive details.

The results of this magnetic resonance study included dental decay, number of teeth, and gum disease. For each tooth surface that was available, we assessed caries indicators using two metrics: the total of tooth surfaces with decay, missing, and

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TABLE 1	Description of 35 bloc	d and urine biomarker	s in the UK Biobank.
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Phenotype	Abbreviation	Units of measurement	Trait category
Alanine aminotransferase	ALT	U/L	Liver
Albumin	ALB	g/L	Liver
Alkaline phosphatase	ALP	U/L	Bone and Joint
Apolipoprotein A	APOA	g/L	Cardiovascular
Apolipoprotein B	APOB	g/L	Cardiovascular
Aspartate aminotransferase	AST	U/L	Liver
AST to ALT ratio	AST2ALT	N/A	Liver
C-reactive protein	CRP	mg/L	Cardiovascular
Calcium	CA	mmol/L	Bone and Joint
Cholesterol	CHOL	mmol/L	Cardiovascular
Creatinine	CRE	µmol/L	Renal
Creatinine in urine	UCR	μmol/L	Renal
Cystatin C	CYS	mg/L	Renal
Direct bilirubin	BILD	µmol/L	Liver
eGFR	EGFR	mL/min/1.73 m <sup>2</sup>	Renal
Gamma glutamyltransferase	GGT	U/L	Liver
Glucose	GLU	mmol/L	Diabetes
HbA1c	HBA1C	mmol/mol	Diabetes
HDL cholesterol	HDL	mmol/L	Cardiovascular
IGF-1	IGF1	nmol/L	Hormone
LDL cholesterol	LDLD	mmol/L	Cardiovascular
Lipoprotein A	LPA	nmol/L	Cardiovascular
Microalbumin in urine	URMA	mg/L	Renal
Non-albumin protein	NAP	g/L	Renal
Phosphate	PHOS	mmol/L	Renal
Potassium in urine	URK	mmol/L	Renal
SHBG	SHBG	nmol/L	Hormone
Sodium in urine	URNA	mmol/L	Renal
Testosterone	TES	nmol/L	Hormone
Total bilirubin	TBIL	µmol/L	Liver
Total protein	ТР	g/L	Renal
Triglycerides	TRIG	mmol/L	Cardiovascular
Urate	UA	µmol/L	Renal
Urea	BUN	mmol/L	Renal
Vitamin D	VITD	nmol/L	Bone and Joint

Abbreviation: eGFR, estimated glomerular filtration rate.

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fillings (DMFS) and the total of tooth surfaces with decay and fillings (DFSS). In individuals of European descent, a recent metaanalysis of GWAS was conducted to acquire summary data on DMFS, DFSS, the number of teeth, and periodontitis.<sup>28</sup> The Genetic Lifestyle Interactions in Dentistry Endpoints (GLIDE) consortium performed a GWAS meta-analysis, which involved 9 studies for the initial examination of DMFS (n = 26,792), 8 for DFSS (n = 26,533), 9 for N teeth (n = 27,949), and 7 for periodontitis (17,353 cases and 28,210 controls). Clinical dental records provided the data for DMFS and DFSS. Clinical dental records were used to collect data for N teeth in all studies, except for one study where self-reporting was used. Periodontitis was diagnosed using the Centers for Disease Control and Prevention/ American Academy of Periodontology definition or similar criteria.<sup>29</sup> Table 2 displays comprehensive details for every study.

### 2.3 | Statistical analysis

The relationship between the concentrations of blood/urine and outcomes was primarily assessed using two-sample MR analysis with the IVW method, which relies on inverse variance weighting. The IVW approach is a perfect estimation and an efficient analysis assuming that all genetic variants act as effective instrumental variables, demonstrating a robust capability to identify causality.<sup>24</sup> However, the IVW method specifically mandates that genetic variations solely impact the desired result via the studied exposure. Despite the exclusion of identified confounding SNPs to the best of our ability, numerous undisclosed confounding variables remain, which impact gene pleiotropy and introduce bias in the estimation of effect values. To ensure the reliability and stability of the findings, two other techniques, specifically MR-Egger regression<sup>30</sup> and the weighted median method (WME),<sup>31</sup>

TABLE 2	Description	of dental	caries	and	periodontitis

Study	Full name	No with GWAS in paper	Age
ARIC	Atherosclerosis Risk in Communities	DMFS (4409) DFSS (4409) N teeth (4409) Periodontitis (4655)	45-64 years
COHRA1	The Center for Oral Health in Appalachia cohort 1 (COHRA1) part of GENEVA caries	DMFS (887) DFSS (887) N teeth (887) Periodontitis (711)	18+ years
DRDR	Dental Registry and DNA Repository (DRDR) of the University of Pittsburgh School of Dental Medicine	DMFS (229) DFSS (229) N teeth (229) Periodontitis (0)	17-84 years
MDC	Malmö Diet and Cancer Study	DMFS (842) DFSS (842) N teeth (842) Periodontitis (0)	45-64 years
NFBC 1966	Northern Finland Birth Cohort 1966	DMFS (1483) DFSS (1483) N teeth (1483) Periodontitis (0)	46-47 years
SHIP	Study of Health in Pomerania	DMFS (3362) DFSS (3362) N teeth (3362) Periodontitis (3065)	20-81 years
SHIP-TREND	Study of Health in Pomerania Trend	DMFS (944) DFSS (944) N teeth (944) Periodontitis (879)	20-83 years
TWINGENE	Swedish Twin Biobank	DMFS (2820) DFSS (2820) N teeth (2820) Periodontitis (1944)	46-93 years
WGHS	Women's Genome Health Study	DMFS (0) DFSS (0) N teeth (1353) Periodontitis (22,290)	45+ years

Abbreviation: GWAS, Genome-Wide Association Studies.





FIGURE 1 IVW estimates from 35 blood/urine metabolites on dental caries and periodontal disease. The color of each block represents the IVW-derived p values of every MR analysis. IVW, inverse variance weighted; MR, Mendelian randomization.

were employed for testing. We conducted MR analysis for each metabolite individually. If the three MR models yielded comparable estimates of the causal effect, we deemed the metabolite's causal relationship with dental caries/periodontal disease to be consistent and trustworthy. The findings were analyzed as beta coefficients and 95% confidence intervals (CIs) for DMFS, DFSS, and N teeth per 1 standard deviation (SD) rise in blood/urine levels. To test for significant causality, the IVW analysis was employed, utilizing a stringent multiple hypothesis test threshold of  $p < 3.68 \times 10^{-4}$ . We also focused on metabolites with p values greater than or equal to  $1.03 \times 10^{-4}$  but less than 0.05 as potential risk predictors for caries/periodontal disease. The associations with p values below 0.05 underwent the subsequent examinations for heterogeneity and genetic pleiotropy.

The estimation of causal effects may be biased in the two-sample MR analysis method due to heterogeneity arising from variations in the analysis platform, experimental conditions, enrollment population, and SNP. Thus, in this research, the heterogeneity examination was conducted on the primary IVW analysis technique and MR-Egger regression. If the p value exceeded 0.05 in the test, it indicated the absence of heterogeneity in the incorporated instrumental variables, allowing the disregard of heterogeneity's impact on estimating causal effects. The MR-Egger regression analysis is applicable for assessing the

bias of genetic pleiotropy. The magnitude of pleiotropy can be evaluated by the regression intercept, and the likelihood of pleiotropy decreases as the intercept approaches zero. The study utilized the *p* value from the genetic pleiotropy test to assess the existence of genetic pleiotropy in the analysis. If the *p* value exceeded 0.05, the presence of genetic pleiotropy in the causal analysis was deemed insignificant, and its impact could be disregarded.24

To test the reliability and stability of the results, the MR-Egger regression method, the WME, the simple estimation method based on the plural, and the plural-based weighted estimation method are utilized, in addition to the four methods stated earlier. In addition, sensitivity analysis was conducted using the leave-one-out method in the study. In other words, the metabolites' sensitivity analysis successfully passed both the heterogeneity test and gene multiplicity test, and their p values in the IVW method used for sensitivity analysis were less than 0.05. After eliminating metabolites that had a p value below 0.05 in the IVW technique and successfully passing the heterogeneity and pleiotropy assessments, we excluded each associated SNP and computed the collective impact of the remaining SNPs. To evaluate the impact of individual SNPs on the metabolites, the cumulative influence of the remaining SNPs was computed. The

impact of every single SNP on metabolites was assessed by eliminating each pertinent SNP and computing the collective impact of the remaining SNPs.<sup>32</sup>

### 3 | RESULTS

### 3.1 | Characteristics of the selected SNPs

Out of the 35 metabolites, there were a total of 34,211 SNPs that showed association with *p* values less than  $5 \times 10^{-5}$ . After LD analysis, 3567 independent SNPs were obtained, of which 477 SNPs were associated with at least two metabolites. Out of the 477 SNPs, there was a single SNP (rs174547) in the PhenoScanner database that showed an association with glycemia traits. In the subsequent analysis, 3566 SNPs were included after excluding confounding SNPs. The median number of instrumental variables for each metabolite was 79, and two metabolites (Microalbumin in urine and Potassium in urine) with instrumental variables less than or equal to 3 were excluded from the subsequent analysis.

# 3.2 | Correlation between levels of blood/urine concentrations and the likelihood of developing dental caries and periodontitis

The primary approach employed in this study was the IVW technique to evaluate the causal association between metabolites and dental caries. Three metabolites (C-reactive protein, urine Potassium, and Urea) exhibited potential significant (p < 0.05) causal effects on dental caries, while none of the metabolites remained significant following multiple hypothesis testing ( $p < 3.68 \times 10^{-4}$ ).

The MR study using the IVW method discovered a potential correlation between genetic susceptibility to C-reactive protein and DFFS (β: -0.083; 95% CI: -0.157 to -0.009; p = 0.027). There was a potential correlation between urinary potassium and DMFS, with a  $\beta$  value of -0.980 (95% CI: -1.831 to -0.128; p = 0.024). The concentration of urea showed a correlation with DMFS ( $\beta$ : -0.180; 95% CI: -0.283 to -0.077; p = 0.001) as depicted in Figures 1, 2, and Table 3. In the MR Egger model, there was a positive correlation between Gamma glutamyltransferase, Urate, Urea, and DFSS (Figure 3). The MR Egger model showed a nominal association between sodium levels in urine, Creactive protein, and DMFS. Cholesterol was positively associated with DMFS in the Weighted median model (Table S1). Both the approach of calculating the median with weights and the MR-Egger estimation yielded inconclusive findings and demonstrated an absence of a causal relationship. Although all three MR models did not reach statistical significance, they all had similar effect values in most blood/urine metabolites, probably because the

xnosure	Outcome	Method	N-SNPs		Р
Alanine aminotransferase	DFSS	MR Egger	152	···· <b> </b> ····	0.484312783
Alanine aminotransferase	DFSS	Weighted median	152	1	0.739009087
Alanine aminotransferase	DFSS	Inverse variance weighted	152	1.0.1	0.526845214
Albumin	DESS	MR Egger Weighted median	157	here and	0.785434622
Albumin	DFSS	Inverse variance weighted	157	1	0.090086219
Alkaline phosphatase	DFSS	MR Egger	231	H-O-H	0.766918856
Alkaline phosphatase	DFSS	Weighted median	231	1	0.722706875
Alkaline phosphatase	DFSS	Inverse variance weighted	231	Hel Le L	0.509332845
Apolipoprotein A	DESS	MR Egger Weighted median	342	•••	0.494498596
Apolipoprotein A	DFSS	Inverse variance weighted	342	IOI	0.184720232
Apolipoprotein B	DFSS	MR Egger	174	·· <b>●</b> ··	0.976657134
Apolipoprotein B	DFSS	Weighted median	174	H-O-H	0.912369471
Apolipoprotein B Aspartate aminotransferas	DFSS	Inverse variance weighted	174	HH I	0.617982464
Aspartate aminotransferas	DFSS	MR Egger	172	here and	0.672225006
Aspartate aminotransferas	DESS	Inverse variance weighted	172	1.0.1	0.09436//1/
AST to ALT ratio	DFSS	MR Egger	155	····•	0.662396596
AST to ALT ratio	DFSS	Weighted median	155	H	0.975468901
AST to ALT ratio	DFSS	Inverse variance weighted	155	1-0-1	0.328769967
C-reactive protein	DFSS	MR Egger	140	I++•++I	0.215709505
C-reactive protein	DFSS	Weighted median	140	··••-	0.096624856
2-reactive protein	DESS	Inverse variance weighted	140	<b> </b>	0.026694069
Calcium	DFSS	Weighted median	139	1	0.595785159
Calcium	DFSS	Inverse variance weighted	139	1-0-1	0.888771284
Cholesterol	DFSS	MR Egger	187	· • • • • •	0.562812306
Cholesterol	DFSS	Weighted median	187	1	0.701200529
Cholesterol	DFSS	Inverse variance weighted	187	Hell	0.835069264
Creatinine	DESS	MR Egger Weighted median	239	F	0.975549035
Creatinine	DFSS	Inverse variance weighted	239	HH I	0.093712175
Creatinine in urine	DFSS	MR Egger	17	·	0.894047525
Creatinine in urine	DFSS	Weighted median	17	·····•	0.272889815
Creatinine in urine	DFSS	Inverse variance weighted	17	·····	0.686427823
Dystatin C	DFSS	MR Egger	217	1 <b>-</b> 1	0.092964397
Dystatin C	DFSS	Weighted median	217	<b>-</b>	0.202047911
Jystatin C Direct bilirabin	DESS	Inverse variance weighted MR Eaguer	59	[···•	0.25864052
Direct bilirubin	DFSS	Weighted median	59	h	0.874684607
Direct bilirubin	DFSS	Inverse variance weighted	59	H-	0.745503716
GFR	DFSS	MR Egger	236	les <b>e</b> ssi	0.83192264
GFR	DFSS	Weighted median	236	H-O-H	0.412615228
GFR	DFSS	Inverse variance weighted	236	Hell	0.119329442
Gamma glutamyltransferase	DFSS	MR Egger	213	[··•	0.019337378
Samma glutamyltransferase	DESS	Inverse variance weighted	213	Hel	0.209071406
Glucose	DFSS	MR Egger	81	h	0.288291562
Glucose	DFSS	Weighted median	81	H-+	0.763213459
Glucose	DFSS	Inverse variance weighted	81	H.	0.801526151
lbA1c	DFSS	MR Egger	212	F-0-1	0.634256997
HbA2c	DFSS	Weighted median	212	1-0-1	0.330407831
IDL cholesterol	DESS	MR Fager	191	H•H	0.272226282
IDL cholesterol	DFSS	Weighted median	191	1-0-1	0.493253061
HDL cholesterol	DFSS	Inverse variance weighted	191	Hel	0.657928504
GF-1	DFSS	MR Egger	260	1	0.319351204
GF-2	DFSS	Weighted median	260	F-0-1	0.227140238
GF-3	DFSS	Inverse variance weighted	260	H#1	0.292925823
DL cholesterol	DESS	MR Egger Weighted median	191	1	0.518/54136
.DL cholesterol	DFSS	Inverse variance weighted	191	He I	0.899166432
.ipoprotein A	DFSS	MR Egger	14	1	0.543573451
.ipoprotein A	DFSS	Weighted median	14	·· <b>●</b> ··	0.490735707
.ipoprotein A	DFSS	Inverse variance weighted	14	1- <b>•</b> -1	0.950660805
Microalbumin in urine	DFSS	MR Egger	0		0
Microalbumin in urine	DESS	Inverse variance weighted	2		0.445830185
Non-albumin protein	DFSS	MR Egger	212	[+- <b>●</b> -+]	0.156798092
Non-albumin protein	DFSS	Weighted median	212	F-●-H	0.577380473
Non-albumin protein	DFSS	Inverse variance weighted	212	HOH	0.614237513
Phosphate	DFSS	MR Egger	122	1	0.510747795
Phosphate	DESS	Weighted median	122	1	0.981991426
nospnate Potassium in urine	DESS	MR Foger	3		0.037151341
Potassium in urine	DFSS	Weighted median	3		0.741546382
Potassium in urine	DFSS	Inverse variance weighted	3	······	0.239460313
SHBG	DFSS	MR Egger	191	1	0.739958287
SHBG	DFSS	Weighted median	191	H	0.525639903
SHBG	DESS	Inverse variance weighted	191	·····	0.506987496
Sodium in urine	DESS	With Egger	18		0.97115996
Sodium in urine	DFSS	Inverse variance weighted	18	·····	0.978844418
lestosterone	DFSS	MR Egger	60		0.73594225
l'estosterone	DFSS	Weighted median	60	kk	0.417891505
Festosterone	DFSS	Inverse variance weighted	60	In the second se	0.268930968
iotai bilirubin Fatal bilirubin	DESS	MR Egger	100	F	0.325329734
rotat ollirubin Fotal bilirubin	DESS	Inverse variance weighted	100		0.830470479
Fotal protein	DFSS	MR Egger	188	<b>-</b>	0.225084578
Fotal protein	DFSS	Weighted median	188	1	0.136625692
Fotal protein	DFSS	Inverse variance weighted	188	1-0-1	0.151558861
Triglycerides	DFSS	MR Egger	168	H•H	0.41535746
ringiyeerides Friebusseides	DESS	Weighted median	168	1	0.120754081
Jrate	DESS	MR Foor	108	1	0.864862107
Jrate	DFSS	Weighted median	174	[··●··]	0.127132527
Jrate	DFSS	Inverse variance weighted	174		0.158058919
				-0.6-0.4-0.2 0.0 0.2 0.4 0.6 0.8 1 R(05%(CT)	.0 1.2 1.4 1.6

**FIGURE 2** Estimated causal effect of 35 blood/urine concentrations on DFSS using different MR methods. MR, Mendelian randomization.

IVW method had higher test power than the other two MR models. The intercept p values for the MR-Egger test were all greater than 0.05. No substantial heterogeneity was observed (all p values for Cochran Q > 0.05). The sensitivity analysis, which

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Exposure	Outcome	IVW-derived p value	β	Upper 95% Cls	Lower 95% Cls	Cochran' s Q-derived p value	MR-Egger intercept- derived <i>p</i> value
C-reactive protein	DFSS	0.026	-0.083	-0.156	-0.009	0.444	0.946
Potassium in urine	DMFS	0.024	-0.98	-1.831	-0.128	0.464	0.532
Urea	DMFS	0.001	-0.180	-0.283	-0.077	0.082	0.735
Cystatin C	N teeth	0.048	-0.071	-0.141	-0.001	0.075	0.664
Non- albumin protein	N teeth	0.043	-0.068	-0.134	-0.002	0.23	0.825
Urea	N teeth	0.010	0.12	0.028	0.212	0.468	0.093
Creatinine	Periodontitis	0.036	0.107	0.006	0.208	0.701	0.057
Cystatin C	Periodontitis	0.025	0.126	0.015	0.237	0.159	0.377
eGFR	Periodontitis	0.032	-0.112	-0.215	-0.009	0.857	0.108
Phosphate	Periodontitis	0.039	-0.147	-0.287	-0.007	0.022	0.981
Urea	Periodontitis	0.014	0.1847	0.035	0.333	0.369	0.676

**TABLE 3** Nominal significant Mendelian randomization estimates of the causal relationship between urine/blood metabolites and dental caries/periodontal disease.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; MR, Mendelian randomization.

involved leaving out one SNP at a time, revealed that none of the individual SNPs had a significant impact on the overall outcome (Table S2).

## 3.3 | Blood/urine metabolites and risk of periodontitis

The primary approach used in this study was the IVW method to evaluate the causal connection between metabolites and the number of teeth affected by periodontitis. Nominal significant causal effect values (p < 0.05) were observed for six metabolites (Cystatin C, Non-albumin protein [NAP], Urea, Creatinine, estimated glomerular filtration rate [eGFR], and Phosphate) concerning N teeth/periodontitis. However, none of the metabolites remained significant after multiple hypothesis testing ( $p < 3.68 \times 10^{-4}$ ).

According to the IVW model, the MR study discovered that an increase of 1 *SD* in Cystatin C levels potentially showed a positive correlation with the number of teeth ( $\beta$ : -0.070; 95% CI: -0.141 to -0.001; *p* = 0.048). There was a potential positive correlation between NAP and the number of teeth ( $\beta$ : -0.068; 95% CI: -0.134 to -0.002; *p* = 0.043). Urea showed a potential positive correlation with the number of teeth ( $\beta$ : 0.120; 95% CI: 0.028 to 0.212; *p* = 0.010) as depicted in Figure 4 and Table 3. In the IVM method, there was a potential positive correlation between Creatinine, Cystatin C, eGFR, Phosphate, and Urea with periodontitis. On the other hand, in the MR Egger model, Creatinine, eGFR, and Testosterone showed a positive association with periodontitis (Figure 5 and Table 3). The additional results are shown in Tables S1 and S2. The intercept of the MR-Egger test had a *p* value greater than 0.05. Based on leave-one-out analyses, it was indicated that no single SNP had a significant impact on the overall outcome of N teeth/periodontal disease.

### 4 | DISCUSSION

This study utilized extensive GWAS data available in public databases to investigate the causal links between 35 metabolites found in blood/urine and the likelihood of developing dental caries/periodontal disease. The analysis employed an impartial two-sample Mendelian randomization approach. Nevertheless, despite rigorous quality control measures, there is a lack of compelling evidence suggesting a direct causal link between these blood metabolites and the development of dental caries or periodontal disease.

Despite none of the 35 blood/urine metabolites examined in this research meeting the criteria for multiple hypothesis testing, they yielded 8 potential indicators of the risk of dental caries/ periodontal disease. These include C-reactive protein, Potassium in urine, Urea, Cystatin C, NAP, Creatinine, eGFR, and Phosphate. Out of the eight metabolites, four metabolites (Urea, Creatinine, Method

Alamina and a start former		Mik Egger			0.389930030
Alanine aminouansierase	DMFS	Weighted median	152	I++••••	0.632014286
Alanine aminotransferase	DMFS	Inverse variance weighted	152	11	0.068370836
Albumin	DMFS	MR Egger	157	1	0.498756727
Albumin	DMFS	Weighted median	157	I++ • • • • I	0.522479692
Albumin	DMFS	Inverse variance weighted	157	I-●-I	0.044305506
Alkaline phosphatase	DMFS	MR Egger	231	1-0-1	0.403896933
Alkaline phosphatase	DMFS	Weighted median	231	I- <b>B</b> -I	0.724776991
Alkaline phosphatase	DMFS	Inverse variance weighted	231	lei	0.833780756
Apolipoprotein A	DMFS	MR Egger	342	I- <b>-</b> -I	0.494498596
Apolipoprotein A	DMFS	Weighted median	342	I- <b>-</b> -I	0.438492041
Apolipoprotein A	DMFS	Inverse variance weighted	342	I <b>O</b> I	0.184720232
Apolipoprotein B	DMFS	MR Egger	174	H-O-H	0.976657134
Apolipoprotein B	DMFS	Weighted median	174	1-0-1	0.91149778
Analinoprotein B	DMES	Invariance weighted	174	iei	0.617982464
Aspartate aminotransferas	DMFS	MD Energy	174		0.01/982404
Aspartate aminotransferas	DMFS	MR Egger	172	L	0.672225006
Aspartate aminotransferas	DMFS	Weighted median	172		0.683950719
e	DMFS	Inverse variance weighted	172	1-1	0.22532879
AST to ALT ratio	DMFS	MR Egger	155	1	0.662396596
AST to ALT ratio	DMFS	Weighted median	155	·· <b>●</b> ··	0.974568125
AST to ALT ratio	DMFS	Inverse variance weighted	155	I- <b>●</b> -I	0.328769967
C-reactive protein	DMFS	MR Egger	140	F-O-H	0.01255317
C-reactive protein	DMFS	Weighted median	140	F-O-H	0.077296103
C-reactive protein	DMFS	Inverse variance weighted	140	He-H	0.109338751
Calcium	DMFS	MR Egger	139	1 <b>-</b> 1	0.732936666
Calcium	DMFS	Weighted median	139	I	0.597773848
Calcium	DMFS	Inverse variance weighted	139	I-O-I	0.888771284
Cholesterol	DMES	MR Fager	187		0 159307224
Cholastarol	DMES	Waightad madian	187	JJ	0.03996563
Cholesterol	DMES	weighted median	107		0.05990303
Cholesteror	DMFS	inverse variance weighted	10/	lu eul	0.008431132
Creatinine	DMFS	MR Egger	239	1	0.975549035
Creatinine	DMFS	Weighted median	239		0.487780497
Creatinine	DMFS	Inverse variance weighted	239	H <b>H</b>	0.093712175
Creatinine in urine	DMFS	MR Egger	17	••••••	0.94140721
Creatinine in urine	DMFS	Weighted median	17	+	0.781776232
Creatinine in urine	DMFS	Inverse variance weighted	17	·····	0.589605389
Cystatin C	DMFS	MR Egger	217	1	0.482867528
Cystatin C	DMFS	Weighted median	217	E-O-A	0.592109759
Cystatin C	DMES	Inverse variance weighted	217	F <b>e</b> -I	0.790503523
Direct hilimbin	DMES	MP Equar	50	1	0.093146172
Direct bilinghin	DMES	Weighted median	50	1	0.150021448
Direct billiobil	DMFS	weighted median	59	1.0.1	0.130921448
Direct bilirubin	DMFS	Inverse variance weighted	59		0.734705547
eGFR	DMFS	MR Egger	236	P	0.440721434
eGFR	DMFS	Weighted median	236	H-0-1	0.447415818
eGFR	DMFS	Inverse variance weighted	236	He-H	0.259310568
Gamma glutamyltransferase	DMFS	MR Egger	213	F-•1	0.072940276
Gamma glutamyltransferase	DMFS	Weighted median	213	I- <b>-</b> -I	0.140786724
Gamma glutamyltransferase	DMFS	Inverse variance weighted	213	I <b>O</b> I	0.189011435
Glucose	DMFS	MR Egger	81	[···•	0.710984763
Glucose	DMFS	Weighted median	81	1	0.26757804
Glucose	DMFS	Inverse variance weighted	81	1-0-1	0.274859687
UbAlo	DMES	MP Equar	212	F	0.721053052
ULA2.	DMES	Weighted median	212	1-0-1	0.520467074
H6A20	DMFS	weighted median	212		0.0710407974
HDASC	DMFS	Inverse variance weighted	212		0.692134401
HDL cholesterol	DMFS	MR Egger	191	1.01	0.181109717
HDL cholesterol	DMFS	Weighted median	191	1-0-1	0.205795981
HDL cholesterol	DMFS	Inverse variance weighted	191	H <del>O</del> H	0.938836441
IGF-1	DMFS	MR Egger	260	··•	0.098475403
IGF-2	DMFS	Weighted median	260	I- <b>e</b> -I	0.484660097
IGF-3	DMFS	Inverse variance weighted	260	Hei	0.797033314
LDL cholesterol	DMFS	MR Egger	191	1-0-1	0.148914658
LDL cholesterol	DMFS	Weighted median	191	1-0-1	0.950788505
LDL cholesterol	D1 (D2	Inverse variance weighted			
**	DMFS	THE STORE FULLWIDE FOR STREET	191	iei	0.400116031
Liboprotein A	DMFS	MR Egger	191	HH 	0.400116031 0.997008432
Lipoprotein A	DMFS	MR Egger Weighted median	191 14 14	₩₩1  @   @	0.400116031 0.997008432 0.976398266
Lipoprotein A Lipoprotein A	DMFS DMFS DMFS DMFS	MR Egger Weighted median	191 14 14	₩1  ₩   -₩   -₩-4	0.400116031 0.997008432 0.976398266 0.968641043
Lipoprotein A Lipoprotein A Lipoprotein A	DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted	191 14 14 14	1⊕1  -⊕1  -⊕-1  -⊕-1	0.400116031 0.997008432 0.976398266 0.968641943
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine	DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger	191 14 14 14 0	H⊕E I+⊕++1 H+⊕+1 H+⊕+1	0.400116031 0.997008432 0.976398266 0.968641943 0
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine	DMFS DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger Weighted median	191 14 14 0 0	101  -01  -0-1  -0-1  -0-1	0.400116031 0.997008432 0.976398266 0.968641943 0 0
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Microalbumin in urine	DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted	191 14 14 0 0 2		0.400116031 0.997008432 0.976398266 0.968641943 0 0 0.278247405
Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Microalbumin in urine Non-albumin protein	DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger	191 14 14 0 0 2 212		0.400116031 0.997008432 0.976398266 0.968641943 0 0 0.278247405 0.635309403
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Non-albumin protein Non-albumin protein	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	M Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median	191 14 14 0 0 2 212 212 212		0.400116031 0.97008432 0.966641943 0 0.278247405 0.635309403 0.584693881
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Non-albumin protein Non-albumin protein	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	M Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted	191 14 14 0 0 2 212 212 212 212		0.400116031 0.997008432 0.976398266 0.968641943 0 0.278247405 0.635309403 0.545493881 0.793164721
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Non-albumin protein Non-albumin protein Phosphate	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger	191 14 14 0 2 212 212 212 212 212 122	104  -∞-1  -∞-1  -∞-1  -∞-1  -∞-1  -∞-1  -0-1	0.400116031 0.997008432 0.976398266 0.968611943 0 0.278247405 0.635309403 0.584693881 0.793164721 0.144349233
Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Microalbumin in urine Non-albumin protein Non-albumin protein Phosphate Phosphate	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MRE gger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median	191 14 14 0 2 212 212 212 212 122 122	104  -∞-1  -∞-1  -∞-1  -∞-1  -∞-1  01  -∞-1	0.400116031 0.997008432 0.976398266 0.968641943 0 0.278247405 0.635309403 0.584693881 0.793164721 0.144349233 0.88661041
Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Microalbumin protein Non-albumin protein Non-albumin protein Phosphate Phosphate	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted Inverse variance weighted	191 14 14 0 2 212 212 212 212 212 122 122 122	00    -∞-1   -∞-1   -∞-1   -∞-1   -∞-1   -∞-1   -∞-1   -∞-1	0.400116031 0.997008432 0.976398266 0.968641943 0.0278247405 0.035309403 0.278247405 0.035309403 0.586403881 0.793164721 0.144349233 0.88641041 0.484350307
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Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin in urine Microalbumin urine Non-albumin protein Non-albumin protein Non-albumin protein Pinophate Pinophate Pinophate Pinophate Pinostasium in urine Potassium in urine	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	Mrk Beger Mrk Beger Weighted median Inverse variance weighted Mrk Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger Weighted median Inverse variance weighted MR Egger	191 14 14 0 2 212 212 212 212 122 122 122 3 3 3 3		0.400116031 0.97008412 0.976398260 0.966641943 0.966641943 0.078247065 0.635350403 0.9546403881 0.793164721 0.14349233 0.89641041 0.485200307 0.462287366 0.022075857
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Lipoprotein A Lipoprotein A Lipoprotein A Microalbumin in urine Microalbumin urine Microalbumin urine Non-albumin protein Non-albumin protein Phosphate Phosphate Phosphate Phosphate Phosphate Stassium in urine Potassium in urine Potassium in urine SHIBG	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	Mrk Berger MR, Begger Weighted median Inverse variance weighted MR, Egger Weighted median Inverse variance weighted MR, Egger Weighted median Inverse variance weighted MR, Egger Weighted median Inverse variance weighted MR, Egger Weighted median Inverse variance weighted MR, Egger	191 14 14 14 0 0 2 212 212 212 122 122 122		0.400116031 0.97008432 0.976398266 0.966841943 0.966841943 0.078347405 0.635309403 0.98649381 0.793164721 0.41439233 0.89641041 0.485200307 0.42287366 0.02267587 0.02607587 0.02607587
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Lapoptotin A Lapoptotin A Lapoptotin A Microalbamin urine Microalbamin urine Microalbamin urine Non-albamin protein Phosphate Phosphate Phosphate Phosphate Phosphate Phosphate Phosphate Station StiBG StiBG Solium in urine Solium in urine	DMFS DMFS DMFS DMFS DMFS DMFS DMFS DMFS	MR Eiger MR Eiger Weighted median Inverse variance weighted MR Eiger	191 14 14 14 0 2 212 212 212 122 122 122 122 122 122	Image: 1	0.400116031 0.97008432 0.976398266 0.968641943 0.068641943 0.0738247405 0.635350403 0.958645881 0.7793164721 0.14347233 0.48530307 0.04673587 0.04673587 0.04673587 0.04673587 0.04673587 0.04673587 0.04673587 0.04673587 0.0475357 0.04757547 0.0475757547 0.04757547547 0.04757547547 0.04757547 0.04757547
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**FIGURE 3** Estimated causal effect of 35 blood/urine concentrations on DMSS using different MR methods. MR, Mendelian randomization.

Cystatin C, and Urea) could potentially correlate with a higher susceptibility to periodontal disease. It was observed that the non-survivor group had elevated levels of high-sensitivity Creactive protein compared to the survivor group. Additionally, YIN ET AL.

during the 3-year follow-up period, the T3 group exhibited a significantly higher mean hs-CRP value than the T1-2 group.<sup>33</sup> To examine the impact of treatment with silver diamine fluoride (SDF) and potassium iodide (KI) on secondary caries, the authors and others conducted an investigation. It was shown that the treatment with SDF + KI decreased the occurrence of secondary caries.<sup>34</sup> In addition, urea was linked to the possibility of tooth decay<sup>35,36</sup> and gum disease.<sup>37,38</sup> The levels of the protein Cystatin C (CSTC), an inhibitor of cysteine protease, and the expression of the CST3 gene were notably elevated in individuals with periodontal disease compared to the healthy population.<sup>39</sup> Additionally, a direct association was observed between the levels of the gene and protein. Urea, Creatinine, and eGFR are all important biomarkers of renal function. Several studies have reported that periodontitis is closely related to chronic kidney disease.<sup>40-43</sup> Dysregulated Phosphate Metabolism was closed with Periodontal Disease.<sup>44</sup> The urinary protein consists of both NAPs and albumin. NAPs consist of small proteins, such as mucoproteins (primarily Tamm-Horsfall protein), blood-group proteins, immunoglobulins, mucopolysaccharides, hormones, and enzymes.<sup>45</sup> There are no reports on caries and periodontal disease for NAPs.

This research possesses several strengths. First, it investigates the causal connection between metabolites in blood/urine and the risk of dental caries/periodontal disease from a molecular mechanism perspective, which holds significant clinical research value and is supported by a strong theoretical foundation. Secondly, the study ensures reliability and stability by implementing rigorous quality control measures, analytical methods, and multiple models to evaluate causal effects. Last, unlike previous Mendelian randomization studies that focused on a single exposure factor, this research tackles numerous metabolites in blood, which presents a substantial workload and demands challenging analysis. This study has certain constraints. First, the GWAS data for periodontal disease, caries, and metabolites were collected from European populations. Therefore, it is necessary to conduct more extensive studies involving diverse ethnic groups. Additionally, despite utilizing the most extensive GWAS data available, future research should aim to include larger sample sizes to obtain a more precise evaluation of the genetic influence of metabolites. Despite utilizing the most extensive GWAS data available, additional research is required to offer a more precise evaluation of the genetic influence of metabolites.

To sum up, we employed a two-sample Mendelian randomization method to investigate the causal connections among 35 blood and urine metabolites and caries as well as periodontal disease. While no strong causal link was established between these blood/urine metabolitesand the risk of caries and periodontal disease, this study's findings on potential predictors of caries and periodontal disease risk offer fresh perspectives on the influence of genetic-exposure interactions in the development of these conditions.

Exposure	Outcome	Method	N.SNPs		Р
Alanine aminotransferase	N teeth	MR Egger	152	······	0.775692661
Alanine aminotransferase	N teeth	Weighted median	152	·····	1
Alanine aminotransferase	N teeth	Inverse variance weighted	152	[ <b>e</b> ]	0.514936245
Albumin	N teeth	MR Egger	157	·····	0.270568399
Albumin	N teeth	Weighted median	157	·····	0.497290596
Albumin	N teeth	Inverse variance weighted	157	h	0.078862587
Alkaline phosphatase	N teeth	MR Egger	231	h	0.50933518
Alkaline phosphatase	N teeth	Weighted median	231	I <b>e</b> I	0.594387919
Alkaline phosphatase	N teeth	Inverse variance weighted	231	F-●-1	0.964797892
Apolipoprotein A	N teeth	MR Egger	342	···•	0.818882345
Apolipoprotein A	N teeth	Weighted median	342	··· <b>•</b> ···	0.482587747
Apolipoprotein A	N teeth	Inverse variance weighted	342	F-●-4	0.188005677
Apolipoprotein B	N teeth	MR Egger	174	h	0.320597885
Apolipoprotein B	N teeth	Weighted median	174	<u>}</u> €4	0.4650819
Apolipoprotein B	N teeth	Inverse variance weighted	174	· •●- ·	0.28793838
Aspartate aminotransferase	N teeth	MR Egger	172	······	0.377773388
Aspartate aminotransferase	N teeth	Weighted median	172	h	0.938801702
Aspartate aminotransferase	N teeth	Inverse variance weighted	172	F <b>●</b> 4	0.280041217
AST to ALT ratio	N teeth	MR Egger	155	······	0.875887092
AST to ALT ratio	N teeth	Weighted median	155	·····	0.400497741
AST to ALT ratio	N teeth	Inverse variance weighted	155	···•	0.573910024
C-reactive protein	N teeth	MR Egger	140		0.45066665
C-reactive protein	N teeth	Weighted median	140	[····•	0.792557524
C-reactive protein	N teeth	Inverse variance weighted	140	h	0.36508367
Calcium	N teeth	MR Egger	139	} <b>{</b>	0.292725753
Calcium	N teeth	Weighted median	139	·····	0.617451203
Calcium	N teeth	Inverse variance weighted	139	H	0.883090591
Cholesterol	N teeth	MR Egger	187	} <b>6</b> 4	0.349202106
Cholesterol	N teeth	Weighted median	187	··· <b>●</b> ···	0.614127875
Cholesterol	N teeth	Inverse variance weighted	187	F-●-1	0.208449786
Creatinine	N teeth	MR Egger	239	·····	0.96728621
Creatinine	N teeth	Weighted median	239		0.746239832
Creatinine	N teeth	Inverse variance weighted	239	[··•	0.948576414
Creatinine Creatinine in urine	N teeth	MR Eagar	17		0.948570414
Creatinine in unite	Niceth	Wichted media	17	· · · · · · · · · · · · · · · · · · ·	0.490975000
Creatinine in urine	N teeth	weighted median	17		0.621249108
Creatinine in urine	N teeth	Inverse variance weighted	17		0.668758388
Cystatin C	N teeth	MR Egger	217		0.176052906
Cystatin C	N teeth	Weighted median	217		0.47640585
Cystatin C	N teeth	Inverse variance weighted	217	[··●··]	0.048029381
Direct bilirubin	N teeth	MR Egger	59		0.303286927
Direct bilirubin	N teeth	Weighted median	59	·····	0.3837789
Direct bilirubin	N teeth	Inverse variance weighted	59	I•	0.512416312
eGFR	N teeth	MR Egger	236	·····	0.850182575
eGFR	N teeth	Weighted median	236	h	0.755241799
eGFR	N teeth	Inverse variance weighted	236	I+•●••I	0.713624212
Gamma glutamyltransferase	N teeth	MR Egger	213	}····-ŧ	0.330490505
Gamma glutamyltransferase	N teeth	Weighted median	213	}···•	0.54983997
Gamma glutamyltransferase	N teeth	Inverse variance weighted	213	<b>●</b>	0.367354072
Glucose	N teeth	MR Egger	81	······	0.668347646
Glucose	N teeth	Weighted median	81	·····	0.455027878
Glucose	N teeth	Inverse variance weighted	81		0.425513098
HbA1c	N teeth	MR Egger	212	···· <b> </b>	0.055734209
HbA2c	N teeth	Weighted median	212	[···•	0.030359973
HbA3c	N teeth	Inverse variance weighted	212	F-O-1	0.381716264
HDL sholastarol	N teeth	MP Eggar	191	h	0.531270810
HDL sholesterol	N teeth	Wiektad madian	191		0.551279819
HDL cholesterol	N teeth	Inverse variance weighted	191	1	0.000099385
ICE 1	Nteeth	MB Eases	191		0.302684412
KGF-1	Niceth	MR Egger	260	have been determined	0.303084413
IGF-2	N teeth	weighted median	260		0.76198075
IGF-3	N teeth	Inverse variance weighted	260		0.612729065
LDL cholesterol	N teeth	MR Egger	191		0.787221273
LDL cholesterol	N teeth	Weighted median	191		0.879300909
LDL cholesterol	N teeth	Inverse variance weighted	191	F	0.476955393
Lipoprotein A	N teeth	MR Egger	14		0.175838994
Lipoprotein A	N teeth	Weighted median	14		0.974054297
Lipoprotein A	N teeth	Inverse variance weighted	14	[]	0.475710712
Microalbumin in urine	N teeth	MR Egger	0	•	0
Microalbumin in urine	N teeth	Weighted median	0	•	0
Microalbumin in urine	N teeth	Inverse variance weighted	2	••••••	0.923673834
Non-albumin protein	N teeth	MR Egger	212	·····	0.419826505
Non-albumin protein	N teeth	Weighted median	212	•••• <b>•</b> ••••	0.113579874
Non-albumin protein	N teeth	Inverse variance weighted	212	1+- <b>-</b> +-1	0.043081163
Phosphate	N teeth	MR Egger	122	·····	0.627621147
Phosphate	N teeth	Weighted median	122	+ <b>-</b>	0.62765608
Phosphate	N teeth	Inverse variance weighted	122		0.37561048
Potassium in urine	N teeth	MR Egger	3		0.363222421
Potassium in urine	N teeth	Weighted median	3	••••••	0.974650442
Potassium in urine	N teeth	Inverse variance weighted	3	••••	0.749150128
SHBG	N teeth	MR Egger	191		0.178497783
SHBG	N teeth	Weighted median	191	h	0.133028179
SHBG	N teeth	Inverse variance weighted	191	+•●••	0.123930044
Sodium in urine	N teeth	MR Egger	18	<u>}</u> }	0.178497783
Sodium in urine	N teeth	Weighted median	18	here the second se	0.139353998
Sodium in urine	N teeth	Inverse variance weighted	18	<b>●</b>	0.123930044
Testosterone	N teeth	MR Egger	60		0.9563102
Testosterone	N teeth	Weighted median	60		0.857071519
Testosterone	N teeth	Inverse variance weighted	60		0.91036093
Total bilirubin	N teeth	MR Egger	100	h	0.152769936
Total bilirubin	N teeth	Weighted median	100	HH	0.640204252
Total bilirubin	N teeth	Inverse variance weighted	100	··· <b>●</b> ···	0.865027139
Total protein	N teeth	MR Eager	188	······	0.361614734
Total protein	N teeth	Weighted median	189		0.91850497
Total protein	N teath	Inverse variance weighted	199		0.120156805
Trialveerides	N texth	MR Farmer	100		0.052522129
Trialuaaridas	Ntech	Wainbtad	100	i	0.032332128
Trighteendes	Nieeth	weighted median	108	i v i	0.029583782
Lista	in teeth	inverse variance weighted	105	i = 10 	0.929/15415
Unite	in teeth	Mik Egger	174	1 · · · · · · · · · · · · · · · · · · ·	0.508142827
Urate	N teeth	weighted median	174		0.990227537
Urate	N teeth	Inverse variance weighted	174		0.186276903
				$-0.4 -0.2 0.0 0.2 0.4 0.6 0.8 1  \beta(95\%CI)$	.0 1.2

**FIGURE 4** The causal effect of 35 blood/urine concentrations on N teeth was estimated using different MR methods. MR, Mendelian randomization.

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Alapina aminotransformed	Pariodontitis	MR Eagar	152		0.221252612
Alanine aminouransierase	Periodonuus	MIK Egger	132	lu <b>e</b> ut	0.321332012
Alanine aminouansierase	renodonuus	weighted median	132		0.62078121
Alanine aminotransferase	Periodontitis	Inverse variance weighted	152		0.474381746
Albumin	Periodontitis	MR Egger	157	····	0.812773491
Albumin	Periodontitis	Weighted median	157	···•	0.855204422
Albumin	Periodontitis	Inverse variance weighted	157	F-O-H	0.785752305
Alkaline phosphatase	Periodontitis	MR Egger	231	1	0.841768858
Alkaline phosphatase	Periodontitis	Weighted median	231	[+• <b>•</b> ••]	0.547006881
Alkalina phasphatasa	Pariodontitio	Inverse verience weichted	221	11	0.261311604
Atkanne prosphatase	Periodoliuus	inverse variance weighted	231	1	0.201311094
Apolipoprotein A	Periodontitis	MR Egger	342		0.855632065
Apolipoprotein A	Periodontitis	Weighted median	342	I++-	0.828095554
Apolipoprotein A	Periodontitis	Inverse variance weighted	342	• <b>●</b> -	0.415997893
Apolipoprotein B	Periodontitis	MR Egger	174	h 🔴 H	0.780637902
Apolipoprotein B	Periodontitis	Weighted median	174	··•	0.659478497
Anolinoprotein B	Periodontitis	Inverse variance weighted	174	1-0-1	0 719954899
Aspartate aminotransferas	Periodoliuus	Inverse variance weighted	174	h	0.717954899
Sanartate aminotransferas	Periodontitis	MR Egger	172		0.210758809
s spartate anniotransieras	Periodontitis	Weighted median	172	F	0.391066913
Aspartate aminotransferas	Periodontitis	Inverse variance weighted	172	++ <b>e</b>	0.212486259
AST to ALT ratio	Periodontitis	MR Egger	155	·····	0.588447647
AST to ALT ratio	Periodontitie	Weighted median	155	b	0.511560200
AST to ALT failo	Periodoliuus	weighted median	155		0.511509299
AST to ALT ratio	Periodontitis	Inverse variance weighted	155	1	0.52353359
C-reactive protein	Periodontitis	MR Egger	140	I+++	0.189012069
C-reactive protein	Periodontitis	Weighted median	140	···•	0.471474987
C-reactive protein	Periodontitis	Inverse variance weighted	140	}- <b>●</b> -1	0.684524257
Calcium	Periodontitis	MR Fager	130	k	0.450911435
Calairea	Dania densisia	Weighted median	120	1	0.967160472
Calcium	renodonuus	weighted median	139		0.807139473
Calcium	Periodontitis	Inverse variance weighted	139	P	0.521620921
Cholesterol	Periodontitis	MR Egger	187	[+ • <b>●</b> • •]	0.137227434
Cholesterol	Periodontitis	Weighted median	187	I+++++	0.181822359
Cholesterol	Periodontitis	Inverse variance weighted	187	H•H	0.203471379
Creatinine	Periodontitie	MR Foor	239	····	0.009136791
Constant	Desis desside	Weisherd and in	200	lu aut	0.3370(7504
Creatinine	Periodonutis	weighted median	239		0.337067384
Creatinine	Periodontitis	Inverse variance weighted	239	1	0.036916251
Creatinine in urine	Periodontitis	MR Egger	17	•	0.96330527
Creatinine in urine	Periodontitis	Weighted median	17	F	0.596826689
Creatinine in urine	Periodontitis	Inverse variance weighted	17	·····	0.723979186
Contation C	Devie develation	MD E	217	keen and	0.06276722
Cystatin C	renodonuus	MIK Egger	217		0.06373723
Cystatin C	Periodontitis	Weighted median	217		0.293338865
Cystatin C	Periodontitis	Inverse variance weighted	217	1-0-1	0.025307029
Direct bilirubin	Periodontitis	MR Egger	59	F	0.47769777
Direct hilimbin	Periodontitis	Weighted median	59	h	0.405608289
Direct bindom	Pariodonnus	weighten mental	59	lu <b>e</b> ul	0.405000205
Direct bilirubin	Periodontitis	inverse variance weighted	59		0.206444491
eGFR	Periodontitis	MR Egger	236	·····	0.018129975
eGFR	Periodontitis	Weighted median	236	ł <b>e</b> ł	0.420440583
eGFR	Periodontitis	Inverse variance weighted	236	1-0-1	0.03292825
Gamma elutamyltransferase	Periodontitis	MR Feger	213	I	0.693111407
Gamma glutamultransforaça	Pariodontitis	Waighted median	212	1	0.591573795
Gamma giutaniyitransierase	renodonnus	weighted median	213		0.581575785
Gamma glutamyltransferase	Periodontitis	Inverse variance weighted	213		0.746940795
Glucose	Periodontitis	MR Egger	81	P4	0.09569229
Glucose	Periodontitis	Weighted median	81	····•	0.059536071
Glucose	Periodontitis	Inverse variance weighted	81	11	0.416145705
HbAlc	Periodontitis	MR Egger	212	F	0.463164238
HbA2c	Pariodontitie	Waighted madian	212	I	0.050682251
H0A2C	Periodonuus	weighted median	212		0.030082231
HbA3c	Periodontitis	Inverse variance weighted	212	1-	0.073334041
HDL cholesterol	Periodontitis	MR Egger	191	I++	0.56998871
HDL cholesterol	Periodontitis	Weighted median	191	I	0.84980498
HDL cholesterol	Periodontitis	Inverse variance weighted	191	I- <b>e</b> -I	0.066308006
IGE-1	Periodontitie	MR Enger	260	I	0.728325861
ICE 2	Denie densiele	Weighted and de	260	ha na sa	0.600168033
101-2	Periodonuus	weighted median	260		0.089138933
IGF-3	Periodontitis	Inverse variance weighted	260	1	0.944362244
LDL cholesterol	Periodontitis	MR Egger	191	1	0.681574268
LDL cholesterol	Periodontitis	Weighted median	191	1	0.618248166
LDL cholesterol	Periodontitis	Inverse variance weighted	191	I- <b>O</b> -I	0.219833987
Linematein A	Dania dontitio	MB Errore	14	been and	0.780220702
Elpoprotein A	Teriodoliudis	MicEgge	14		0.109229105
Lipoprotein A	Periodontitis	weighted median	14		0.7225853
Lipoprotein A	Periodontitis	Inverse variance weighted	14	I <b>-</b> I	0.956290161
Microalbumin in urine	Periodontitis	MR Egger	0	•	0
Microalbumin in urine	Periodontitis	Weighted median	0	•	. 0
Microalbumin in urine	Periodontitis	Inverse variance weighted	2	·	0.363180772
Non-albumin protein	Periodontitie	MR Forer	212	h	0.578576694
Non-albumin protein	Periodontiti-	Weighted modion	212	h	0.202422214
New allowing protein	Paris 2	incignica inculari	212	1.00	0.370432214
ison-albumin protein	Periodontitis	inverse variance weighted	212		0.531537216
Phosphate	Periodontitis	MR Egger	122	····	0.245999814
Phosphate	Periodontitis	Weighted median	122	1	0.08929361
Phosphate	Periodontitis	Inverse variance weighted	122	[+ • • • •]	0.039199587
Potassium in urine	Periodontitie	MR Faser	3		0 457996642
Potageium in utine	Dariodontiti	Waiahtad modium	2		0.796430664
a otassium in drine	renodonuus	weighted median	3		0.780429306
Potassium in urine	Periodontitis	inverse variance weighted	3		0.755877039
SHBG	Periodontitis	MR Egger	191	<b> </b>	0.797788833
SHBG	Periodontitis	Weighted median	191	HeH	0.407168771
SHBG	Periodontitis	Inverse variance weighted	191	I- <b>-</b> -I	0.371474492
Sodium in urine	Periodontitie	MR Fooor	18		0 00081839
Codium in unic -	Pariod	Walaktad d'	10		0.333133045
soutum in unifie	renodontitis	weignied median	18		0.372177945
Sodium in urine	Periodontitis	Inverse variance weighted	18	······	0.741136631
Testosterone	Periodontitis	MR Egger	60	F	0.033349461
Testosterone	Periodontitis	Weighted median	60	F	0.465303386
Testosterone	Periodontiti-	Inverse variance weight-1	60	···•	0 160000387
Total bilimbir	Dariad-mili	MP From	100		0.460204462
Total Dilirubin	renodontitis	MR Egger	100		0.469294463
Total bilirubin	Periodontitis	Weighted median	100	h	0.766701429
Total bilirubin	Periodontitis	Inverse variance weighted	100	1++	0.959791999
Total protein	Periodontitis	MR Egger	188	I	0.992232543
Total protein	Pariodontitio	Waiahtad madiar	188	I	0.673385700
Total protein	Periodonuus	weighted median	188	Land I	0.073385709
1 otal protein	Periodontitis	inverse variance weighted	188	r ••• 1	0.336435359
Triglycerides	Periodontitis	MR Egger	168	1	0.912301592
Triglycerides	Periodontitis	Weighted median	168	ł <b>-</b> ł	0.66019598
Triglycerides	Periodontitis	Inverse variance weighted	168	1-0-1	0.072732551
Urate	Periodontitie	MR Faser	174	[···•	0.880209247
Unite	Paris	wax a gger	174	hum <b>e</b> and	0.00020924/
Urate	reriodontitis	Weighted median	174		0.875484407
Urate	Periodontitis	Inverse variance weighted	174		0.511585596
				-1.0-0.8-0.6-0.4-0.20.0 0.2 0.4 0.6 0.8 1	0 1.2 1.4 1.6

**FIGURE 5** The causal effect of 35 blood/urine concentrations on periodontal disease was estimated using different MR methods. MR, Mendelian randomization.

### AUTHOR CONTRIBUTIONS

Xinhai Yin: investigation; validation; writing-original draft. Yadong Wu: resources; software; visualization. Jukun Song: conceptualization; data curation; formal analysis; writing-review & editing. All authors have read and approved the final version of the manuscript

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### CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Jukun Song had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

### TRANSPARENCY STATEMENT

The lead author Jukun Song affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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