Relationship of the Serum CRP Level With the Efficacy of Metformin in the Treatment of Type 2 Diabetes Mellitus: A Meta-Analysis

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Background: Metformin, an anti-diabetes drug, is always used as a first-line agent for the management of T2DM. This meta-analysis was conducted to investigate whether CRP was sensitive in predicting the efficacy of metformin in the treatment of T2DM. **Methods:** Potential relevant studies were identified covering the following databases: MEDLINE, Science Citation Index database, the Cochrane Library Database, PubMed, EMBASE, CINAHL, Current Contents Index, the Chinese Biomedical Database, the Chinese Journal Full-Text Database, and the Weipu Journal Database. Data from eligible studies were extracted and included into the meta-analysis using a random effects model. Statistical analyses were calculated using the version 12.0 STATA software. **Results:** A total of 33 articles including 1,433 subjects were collected for analysis. Pooled SMD of those studies revealed that serum lev-

els of CRP and hs-CRP significantly decreased in patients with T2DM after receiving the metformin treatment. Subgroup analysis by country yielded significant different estimates in the serum levels of CRP between the baseline and after metformin treatment in the China, Israel and India subgroups; but only detected only in the China subgroup considering serum levels of hs-CRP. Follow-up time-stratified analyses indicated that serum levels of CRP were markedly reduced in the metformin-treated group in all subgroups. While differences in serum hs-CRP levels were not observed in two subgroups. **Conclusion:** Decreased serum levels of CRP and hs-CRP may contribute to a more sensitive prediction in providing a more accurate efficacy reference in the metformin drug in T2DM patients. J. Clin. Lab. Anal. 30:13–22, 2016. © 2014 Wiley Periodicals, Inc.

Key words: C-reactive protein; meta-analysis; metformin; type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common metabolic disease that occurs when an individual's body is unable to secrete adequate amounts of insulin or when the insulin produced is unable to be used by the body due to insulin resistance (1, 2). The general signs and symptoms of T2DM are known to be frequent urination, constant hunger, excessive thirst, lack of energy, weight loss or weight gain, and blurred vision (2–4). Globally, as of 2010, there was an estimated 285 million population that had diabetes, with T2DM making up about 90% of all cases of diabetes (5, 6). Furthermore, the incidence and prevalence of T2DM is increasing throughout the world, especially in Westernized societies (6, 7). However,

the countries with the greatest increase in rates of T2DM were expected to be those in Asia and Africa (8). Although the mechanisms of T2DM remain obscure, several genetic and environmental factors are considered to increase the risk of developing T2DM together (9). Some environmental factors are believed to contribute to the development of T2DM, including obesity, increasing

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age, physical inactivity, lack of sleep, high fat, low-fiber diets, smoking, low birth weight, and life stress (9–11). In recent decades, some researches demonstrated that increased physical activity and dietary changes are the initial therapy for T2DM (12, 13). If blood sugar levels are not adequately lowered by these measures, an oral antidiabetes drug (OAD) such as metformin may be used as a first-line agent for the management of T2DM (14, 15). The main function of metformin is known to reduce hepatic glucose production and improve insulin sensitivity, thus metformin may be a valuable agent in improving glycemic control (16, 17). After being treated with metformin, there is a fall in C-reactive protein (CRP) in patients with T2DM, suggesting that CRP may be an effective marker in the assessment of metformin treatment in patients with T2DM (17, 18).

CRP, an annular and pentameric protein found in the blood plasma, is documented to be the first identified pattern recognition receptor (PRR), and has no association with C-peptide or protein C (19, 20). CRP is an acute-phase reactant primarily synthesized in the liver secreted by macrophages and fat cells under the stimulation of adipocyte-derived pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor alpha (21). The levels of CRP in human plasma are indicated to elevate 1,000-fold in response to inflammation, bacterial infections as well as cancers or even after surgical procedures (17). Patients with high serum CRP levels are much more likely to suffer from inflammatory bowel disease, including ulcerative colitis and Crohn's disease, and CRP as a sensitive marker of systemic inflammation, is also promoted in obstructive sleep apnea (22, 23). In addition, patients with elevated levels of CRP have been suggested to be at an increased risk of diabetes as well, especially T2DM (24, 25). There is a hypothesis that physiological function of CRP is to bind to phosphocholine on the exterior of dying or dead cells and some kinds of microbes to activate the complement system, and enhances the opsonin-mediated phagocytosis of macrophages by up-regulating the complement inhibitor factors expression on endothelial cells (26). As a result, CRP takes part in host defense while simultaneously alleviating the potentially damages of complement components in later stage, which results in endothelial dysfunction and probably leads to the chronic inflammation (17, 21). However, patients with T2DM might benefit from the medicine treatment with metformin since metformin has been proven to decrease the levels of CRP according to the previous trials (27, 28). To date, studies referring to the effects of metformin in CRP have presented a similar outcome that CRP may be chosen as a candidate in determining the metformin benefits via calculating serum CRP levels (29, 30); some subsequent trials however failed to find that CRP was use-

ful (31, 32). The present meta-analysis is therefore carried out to provide more lines of evidences in whether CRP is advantageous or not to witness the clinical curative effects of metformin on T2DM.

MATERIALS AND METHODS

This meta-analysis was conducted according to the guidelines of the preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement on the quality of published systematic review and meta-analyses (28).

Search Strategy

Potential relevant studies were identified by a comprehensive literature search without language restriction, which covered the following computerized bibliographic databases: MEDLINE (1966~2014), Science Citation Index database (1945~2014), the Cochrane Library Database (Oxford, UK, Issue 12, 2014), PubMed (1966~2014), Embase (1974~2014), CINAHL (1982~2014), and Current Contents Index (1995~2014). Three Chinese databases (Chinese Biomedical Database, 1978~2014; the Chinese Journal Full-Text Database, 1980~2014; and the Weipu Journal Database, 1989~2014) were also applied to identify Chinese-language articles. We used the following medical subject headings and free language terms in conjunction with a highly sensitive search strategy: diabetes mellitus, type 2 or diabetes mellitus or T2DM and metformin or dimethylguanylguanidine or glucophage or diabex or dimethylbiguanide or melbin or mellitin or hydrochloride and C-reactive protein. Additionally, reference lists of relevant studies selected from the electronic debates were searched manually to find additional work.

Inclusion and Exclusion Criteria

To be included in the systematic review, retrieved studies had to be assessed for their suitability for meeting the following criteria: (1) the search results were conducted within a human population and published in a peer-reviewed journal; (2) only those cohort studies examining the efficacy of metformin monotherapy on the serum levels of hs-CRP and CRP in patients with T2DM were incorporated into the meta-analysis; (3) all patients satisfied the definition and diagnosed criteria of T2DM by the provisional report of a consultation conducted by the WHO (33); (4) the article must present original data and supply sufficient information on the serum levels of hs-CRP and CRP, and follow-up time in patients with

T2DM; (5) the study should provide enough data to calculate an effect size; (6) once studies provided overlapping data, we would choose the study that had the largest number. The major exclusion criteria in this systematic review were as follows: (1) the articles that did not satisfy the current inclusion criteria; (2) some publication types, such as letters, abstracts, reviews, meta-analysis, and proceedings; (3) unpublished sources of data; (4) duplication publications; (5) studies using low-sensitivity hs-CRP/CRP assays (lower limits of assay > 0.5 mg/l). With the help of these inclusion criteria, the title and abstract of all the articles were evaluated on relevance. From the selected articles, the full texts were reviewed, followed by a decision on their eligibility for inclusion.

Study Quality and Data Extraction

Two experienced reviewers independently assessed the methodological quality of the included trials using the critical appraisal skills program (CASP, Milton Keynes Primary Care Trust, 2002, Institute of Health Sciences, Oxford) to ensure consistency in reviewing and reporting results (available at <http://www.phru.nhs.uk/casp/qualitat.htm>).

Each of the two reviewers assessed the studies independently based on the inclusion/exclusion criteria mentioned before the Methods section. We used a standardized data form in duplicate to collect the following descriptive information: surname and initials of the first author, the year of publication or submission, journal, source country, racial descent of study population, language of publication, study design, number of subjects, demographic variables of the subjects, metformin dose, weeks of followup, serum levels measurement, baseline hs-CRP/CRP, changes in hs-CRP/CRP after treatment and confirmation of diagnosis, and so on. Disagreement on the inclusion of a single study was settled by discussion, or a third investigator was consulted.

Statistical Analysis

The standardized mean differences (SMD) for the serum levels of hs-CRP/CRP in patients with T2DM pre- and postmetformin treatment were calculated. A 95% confidence interval (95% CI) was calculated for the summary SMD by the use of Z-test. Also, a test for heterogeneity between trials included for each comparison was performed by the use of the Cochran's Q -statistic and I^2 tests (34). If the Q -test showed evidence of a $P < 0.05$ or I^2 test exhibited $> 50\%$, indicating maximal heterogeneity among the included studies, we did meta-regression analysis with a random-effects model to explore sources of heterogeneity, and otherwise SMDs were pooled in accordance with the fixed-effects model (35, 36). Because

significant heterogeneity existed, the average mean differences in CRP change (and 95% CI) were assessed for subgroups of different explanatory variables. Additionally, in order to evaluate the impact of single studies on the overall estimate, a one-way sensitivity analysis was employed. Further, Egger's linear regression test with visual inspection of the funnel plot was applied to detect the potential publication bias (37, 38). Statistical analyses were conducted with the STATA statistical software (version 12.0, Stata Corporation, College Station, TX).

RESULTS

Description of Included Studies

The combined electronic and manual search initially resulted in 299 potentially eligible articles. After the exception of three duplicated studies, these retrieved studies ($n = 296$) were screened by title and abstract for relevance; subsequently, 150 irrelevant articles were excluded. Then after full-text reading, 111 articles were deemed unsuitable and were therefore excluded, and 35 articles were identified to be included in qualitative analysis. In addition, another two studies were excluded due to lack of data integrity after a more careful assessment of the remaining articles (17, 18, 27–32), (39–63). Flow diagram of study-selection progress and the main reason for exclusion was displayed in Figure 1. Finally, 33 cohort studies composed of 1,433 T2DM cases were incorporated into the current meta-analysis, the sample size of the 33 studies ranged

Fig. 1. Flow chart of literature search and study selection. Thirty-three clinical cohort studies were included in this meta-analysis.

Fig. 2. Distribution of topic-related literature in electronic database over the last decade.

from 12 to 110 participants. All eligible studies were published from 2004~2013 (Fig. 2). All the enrolled papers showed moderate-high quality.

From the 33 included studies, 14 studies included with 610 subjects (range, 12~110) focused on the serum CRP level in patients with T2DM pre- and postmetformin treatment, and the other 19 studies consisted with 823 cases (range, 18~75) concerned about the serum hs-CRP level. All of the 14 studies (CRP) were performed in Asians (China (Xu Q, Zhou Q, Ke XY, Fang M, Zhang H, Tong XZ, Huang CZ, Chen JF, Tian XN, Wang L, and Wang SJ), Korea (Park JS), India (Charkraborty A), and Israel (Farah R)). Three studies failed to obtain both the gender and age information (Xu Q, Zhou Q, and Chen JF), one study did not provide the gender information (Farah R), and another one study lost the age information (Tong XZ). The follow-up duration ranged from 8 to 24 weeks. Of the 19 studies (hs-CRP), 16 studies were from Asians [China (Zhu HL, Zhang Y, Wei LG, Lin X, Yuan ML, Wang SY, Zhu ZL, Zhang YF, Xie S, Li Y, Mao XM, Xu XH, Lai ZF, Gao L, and Yatagai T), India (Kumar R), and Japan (Yatagai T)]; and another three studies were conducted in Caucasians (USA (Stocker DJ), Greece (Kadoglou NP) and Germany (Forst T)). Five studies failed to obtain both the gender and age information (Zhu HL, Zhang Y, Wang SY, Zhang YF, and Xu XH), one study did not provide the gender information (Li Y), and another one study lost the age information (Zhu ZL). The follow-up duration ranged from 6 to 24 weeks. Three studies lost the metformin dose information (Forst T, Li Y, and Lai ZF). In addition, the detection of serum levels of CRP and hs-CRP were performed with enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). Tables 1 and 2 showed the baseline characteristics and serum levels of CRP and hs-CRP pre- and postmetformin treatment of the individual studies.

Quantitative Data Synthesis

The following analyses were performed with a random-effects model for the evidence of Q -test and I^2 test (CRP: $I^2 = 72.3\%$, $P < 0.001$; hs-CRP: $I^2 = 84.2\%$, $P < 0.001$, respectively). Our results showed that serum levels of CRP significantly decreased in patients with T2DM after receiving the metformin treatment compared with the baseline CRP levels, according to the random effects pooled SMD in the five studies (SMD = 0.85, 95%CI = 0.74~0.96, $P < 0.001$). In addition to serum ghrelin levels, findings in the current meta-analysis revealed that serum levels of hs-CRP were markedly reduced in the metformin-posttreated patients in contrast to the metformin-pretreated patients, the pooled SMD of serum hs-CRP levels pre- and postmetformin treatment was (SMD = 0.43, 95%CI = 0.33~0.53, $P < 0.001$; Fig. 3).

In the country-stratified subgroups, the results yielded significant different estimates in the serum levels of CRP

Fig. 3. Forest plots for the differences in serum CRP level with the efficacy of metformin in the treatment of T2DM.

TABLE 1. Characteristics of Included Studies Focused on Protein Expression of CRP

First author	Year	Country	Ethnicity	Sample	Gender (M/F)	Age (years)	Metformin (g/d)	Follow-up (wks)	CRP (mg/l)						NOS score
									Baseline			After treatment			
									N	Mean	SD	N	Mean	SD	
Xu Q (54)	2013	China	Asians	56	—	—	1.50	12	56	1.43	0.31	56	1.28	0.28	6
Zhou Q (61)	2012	China	Asians	46	—	—	1.50	12	46	4.67	0.75	46	3.85	0.82	6
Ke XY (44)	2012	China	Asians	40	21/19	55.4 ± 8.3	0.75~1.50	12	40	6.05	1.23	40	5.16	1.02	7
Fang M (41)	2012	China	Asians	12	8/4	50.3 ± 4.0	1.50	12	12	6.53	4.61	12	3.26	2.79	7
Zhang H (58)	2011	China	Asians	42	22/20	42.6 ± 6.1	1.00	12	42	4.60	2.20	42	3.10	1.60	8
Park JS (32)	2011	Korea	Asians	33	17/33	63.1 ± 8.4	1.00	24	33	1.74	1.07	33	1.52	0.69	8
Charkraborty A (39)	2011	India	Asians	110	61/49	30~55	0.85~2.00	24	110	1.45	0.65	110	0.96	0.23	8
Tong XZ (49)	2009	China	Asians	48	28/20	—	1.50	12	48	7.78	2.16	48	6.42	1.94	7
Huang CZ (31)	2008	China	Asians	25	14/11	40.4 ± 6.6	0.85~1.70	12	25	4.81	0.74	25	4.77	0.16	8
Chen JF (40)	2008	China	Asians	30	—	—	0.75~1.50	4	30	0.89	0.22	30	0.69	0.24	6
					—	—	0.75~1.50	8	30	0.89	0.22	30	0.49	0.23	
Farah R (29)	2008	Israel	Asians	30	—	30~50	0.85	4	30	8.30	1.60	30	6.60	1.10	7
								8	30	8.30	1.60	30	5.80	1.50	
Tian XN (48)	2007	China	Asians	28	16/12	42.6 ± 6.1	1.70	12	28	4.60	2.20	28	3.10	1.60	8
Wang L (30)	2006	China	Asians	26	16/10	47.8 ± 1.8	1.50	24	26	5.90	0.80	26	4.50	0.40	8
Wang SJ (50)	2005	China	Asians	84	52/32	52.8 ± 6.1	0.75~1.50	24	84	5.78	3.80	84	3.78	2.60	8

M, male; F, female; NOS, Newcastle–Ottawa Scale.

between the baseline and after metformin treatment in the China, Israel, and India subgroups (China: SMD = 0.80, 95%CI = 0.66~0.93, $P < 0.001$; India: SMD = 1.01, 95%CI = 0.72~1.29, $P < 0.001$; Israel: SMD = 1.41, 95%CI = 1.01~1.82, $P < 0.001$, respectively), but not in the Korea subgroup (SMD = 0.24, 95%CI = -0.24~0.73, $P = 0.323$). The serum levels of hs-CRP reduced significantly after metformin treatment in comparison to the pretreatment only in the China subgroup (SMD = 0.55, 95%CI = 0.44~0.67, $P < 0.001$), while not observed in

TABLE 2. Characteristics of Included Studies Focused on Protein Expression of Hs-Crp

First author	Year	Country	Ethnicity	Sample	Gender (M/F)	Age (years)	Metformin (g/d)	Follow-up (wks)	hs-CRP (mg/L)						NOS score
									Pretreatment			Posttreatment			
									N	Mean	SD	N	Mean	SD	
Zhu HL(62)	2013	China	Asians	68	—	—	0.75~1.50	8	68	6.71	1.31	68	6.52	1.23	6
Zhang Y(59)	2013	China	Asians	75	—	—	1.00	12	75	2.67	0.43	75	2.03	0.54	6
Wei LG(52)	2013	China	Asians	34	18/16	53.1 ± 8.2	1.00~1.50	12	34	2.48	0.66	34	1.92	0.53	7
Lin X(47)	2013	China	Asians	20	9/11	55.3 ± 9.4	0.85	12	20	2.30	0.61	20	2.24	0.37	7
Kumar R(17)	2013	India	Asians	30	—	53.8 ± 12.2	1.00~1.50	12	30	1.29	1.79	30	1.28	0.09	7
Yuan ML(57)	2012	China	Asians	64	26/28	48.5 ± 8.5	1.00~1.50	12	64	1.78	0.84	64	1.62	0.65	8
Wang SY(51)	2012	China	Asians	75	—	-	0.75~2.00	12	75	2.17	0.88	75	1.64	0.43	6
Forst T(18)	2012	Germany	Caucasians	19	10/9	57.9 ± 5.9		6	19	2.10	2.20	19	2.40	1.70	7
								12	19	2.10	2.20	19	2.30	2.00	
Zhu ZL(63)	2011	China	Asians	33	19/14	—	1.50	24	33	5.75	0.57	33	5.24	0.63	7
Zhang YF(60)	2010	China	Asians	49	—	—	0.75~1.50	12	49	6.77	1.58	49	6.58	1.4	6
Kadoglou NP(43)	2010	Greece	Caucasians	48	16/32	62.7 ± 6.8	0.85~2.50	14	48	2.36	1.02	48	2.15	0.92	8
Xie S(53)	2009	China	Asians	32	18/14	54.5 ± 7.5	1.50	8	32	3.03	0.48	32	1.75	0.39	8
Li Y(46)	2009	China	Asians	48	—	34.1 ± 13.0	24	48	7.08	2.16	48	5.32	1.57	6	
Mao XM(28)	2009	China	Asians	43	23/20	54.1 ± 8.9	0.50~2.50	8	43	2.22	0.67	43	2.26	0.72	8
Xu XH(55)	2008	China	Asians	24	—	—	1.00	12	24	1.67	1.73	24	1.07	1.35	6
Lai ZF(45)	2007	China	Asians	48	20/28	60.2 ± 8.1	12	48	1.66	0.93	48	1.20	0.86	7	
Gao L(42)	2007	China	Asians	48	22/26	56.8 ± 6.0	0.75	12	48	4.71	0.82	48	4.67	0.86	8
Stocker DJ(27)	2007	USA	Caucasians	47	25/22	65.0 ± 10.0	1.75	24	47	5.69	0.83	47	5.67	0.33	8
Yatagai T(56)	2004	Japan	Asians	18	8/7	57.0 ± 3.0	0.75	12	18	1.09	0.25	18	1.15	0.30	8

M, male; F, female; wks, weeks; NOS, Newcastle–Ottawa Scale; CRP, C-reactive protein.

Fig. 4. Subgroup analysis by ethnicity for the differences in serum CRP level with the efficacy of metformin in the treatment of T2DM.

the other five subgroups (all $P > 0.05$). In the follow-up time-stratified subgroups, evidence suggested that serum levels of CRP were markedly reduced in the metformin-treated group compared with the baseline levels in all the follow-up subgroups (4 weeks: SMD = 1.05, 95%CI = 0.66~1.43, $P < 0.001$; 8 weeks: SMD = 1.69, 95%CI =

1.27~2.11, $P < 0.001$; 12 weeks: SMD = 0.69, 95%CI = 0.52~0.85, $P < 0.001$; 24 weeks: SMD = 0.84, 95%CI = 0.66~1.03, $P < 0.001$, respectively). Meanwhile, significant differences in the serum hs-CRP levels were observed before and after metformin treatment in three subgroups (8 weeks: SMD = 0.41, 95%CI = 0.17~0.66, $P = 0.001$;

Fig. 5. Sensitivity analysis of the summary odds ratio coefficients for the differences in serum CRP level with the efficacy of metformin in the treatment of T2DM.

12 weeks: $SMD = 0.45$, $95\%CI = 0.32 \sim 0.57$, $P < 0.001$; 24 weeks: $SMD = 0.56$, $95\%CI = 0.31 \sim 0.81$, $P < 0.001$, while not in the other two subgroup (6 weeks: $SMD = -0.15$, $95\%CI = -0.79 \sim 0.48$, $P = 0.639$; 14 weeks: $SMD = 0.22$, $95\%CI = -0.19 \sim 0.62$, $P = 0.291$, respectively; Fig. 4).

We further conducted sensitivity analyses to determine whether review conclusions were affected by the choice of single study, the finding suggested that no single study had the effect on the pooled SMDs in the current meta-analysis (Fig. 5). Finally, the Egger's regression test showed no evidence of asymmetrical distribution in the funnel-plot in serum levels of CRP in baseline and post-treatment (Egger's test: $t = 1.32$, $P = 0.207$); similar association was also found in changes in the serums

Fig. 6. Funnel plot of publication biases for the differences in serum CRP level with the efficacy of metformin in the treatment of T2DM.

hs-CRP levels (Egger's test: $t = 0.16$, $P = 0.875$) in the systematic reviews (Fig. 6).

DISCUSSION

We performed the current meta-analysis in an attempt to investigate the connection of serum CRP levels with the efficacy of metformin in the management of T2DM patients. Our findings showed that baseline serum CRP levels in patients with T2DM were significantly higher than serum CRP levels after treatment with metformin, demonstrating that measurement of serum CRP levels may be an effective way in evaluating the efficacy of metformin therapy in T2DM patients. CRP, a member of the pentraxin family of plasma proteins, is considered as a positive acute-phase protein consisting of five identical nonglycosylated polypeptide subunits (64). CRP has also been demonstrated to be a systemic marker that is extremely sensitive to inflammatory condition and tissue damage, and plays a crucial part in atherothrombotic cardiovascular disease (65). As a main inflammatory factor,

CRP is generated by the liver during acute infection or inflammation, and its concentration in serum may increase as much as 1,000-fold under the condition of injury and infection (17). In addition, CRP is capable of impairing nitric oxide production directly, leading to endothelial dysfunction (66). Since low-grade systemic inflammation has been supposed to precede and predict the development of both T2DM and atherothrombotic diseases, increased CRP might be an important risk factor in the pathogenesis of T2DM (67). Consequently, it is reasonable to hypothesize that metformin may be effective in the treatment of type 2 diabetes patients for the fact that serum levels of CRP decreased after metformin treatment. In a previous study conducted by Chakraborty et al., a significant reduction in serum CRP concentrations was also observed after metformin therapy (39). Furthermore, the results of our meta-analysis also indicated that serum hs-CRP levels of type 2 diabetes patients after treatment with metformin reduced significantly compared with those before metformin treatment, suggesting that serum hs-CRP levels might be closely connected with metformin treatment for type 2 diabetes patients. Consistent with our results, Pradhan et al. also found that serum hs-CRP levels of type 2 diabetes patients exhibited an obvious reduction after treatment with active metformin (65).

Subgroup analysis by country and follow-up time was implemented in order to deepen our understanding of the relationship between serum CRP levels and the effect of metformin in the treatment of type 2 diabetes patients. In the subgroup analysis based on country, the findings revealed that serum CRP levels after metformin treatment was obviously lower than baseline levels of serum CRP in Chinese, Indians, Israelis, but not in Koreans. However, the correlation between serum hs-CRP levels and the efficacy of metformin treatment for type 2 diabetes patients was shown to be only statistically significant in Chinese. With regard to subgroup analysis stratified by follow-up time, our results indicated that serum CRP levels might be linked to the efficacy of metformin therapy for patients with type 2 diabetes in 4, 8, 12, 24 h after metformin treatment. Nevertheless, serum hs-CRP levels were demonstrated to be correlated with the effect of metformin on T2DM patients only in 8, 12, 24 h after metformin treatment. In summary, the main results of our meta-analysis was in concordance to previous findings that metformin may reduce serum CRP levels of patients with T2DM, illustrating that detection of changes in serum CRP levels may be valuable in assessing the efficacy of metformin therapy in T2DM and predicting the overall survival of T2DM patients.

Sincerely, several advantages should be acknowledged in this meta-analysis. On the one hand, the inclusion of unpublished literatures on CRP and the efficacy of metformin T2DM treatment may weaken the persuasion of

our results. Furthermore, all studies included have an acceptable quality (scored at least seven). Moreover, publication bias was not discovered when using the Egger's test, indicating that the statistic data obtained from those included literatures may approximate the actual results. However, there did exist some limitation in the current meta-analysis that should be taken into consideration. First, publication and reporting bias may be existed. We did not take unpublished papers and abstracts into account because the required data were unavailable for the inclusion and exclusion criteria. A second potential limitation may be that there existed barely standardized criteria in judging the efficacy of metformin in T2DM treatment. Additionally, though comprehensively data were extracted for statistics analysis, studies included in this meta-analysis contained various ethnic populations and nations, and gender, age, lifestyle, culture barriers, especially access to health care and efficacy judgments were all disparate. All of the above information we used could cause an inconsistent outcome. Moreover, another limit may be that ranges of different ethnic background populations were not included in this research, which may contribute to an increased ethnic bias.

In conclusion, decreased serum CRP and hs-CRP level may be closely correlated with a better efficacy of metformin in patients with T2DM in the present meta-analysis. CRP and hs-CRP may be considered as sensitive biomarker for clinical usage of making difficult therapeutic decisions. To confirm our findings, a more adequately designed research with large sample size should be determined by a more appropriate multivariate analysis.

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