Original Article

A study on the recent 20 years' herbal medication rules and syndromes distribution in TCM treatment of Diabetic Nephropathy by Data mining

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Aim: To study the recent 20 years' herbal medication rules and syndromes distribution in TCM treatment of diabetic nephropathy based on modern literature.

Methods: We used SPSS18.0 and SPSS Modeler14.1 for data analysis. The descriptive analysis and cluster analysis were performed by SPSS 18.0; the association rules analysis with the Apriori algorithm was performed by SPSS Modeler14.1.

Results: 274 TCM formulas were obtained after screening. The 5 most frequently used Chinese herbal medicines are milkvetch root, Indian bread, common yam rhizome, danshen root, Asiatic cornelian cherry fruit; The 5 most common syndromes of DN are deficiency Qi and Yin, obstruction of collaterals by blood stasis, deficiency of liver and kidney, damp-heat stasis obstruction, Yin asthenia generating intrinsic heat; the 5 most common pathological factors are Yin deficiency, blood stasis, Qi deficiency, endogenous heat and phlegm. In association rules analysis, we displayed association rules among herbs of confidence greater than 85% and support greater than 15%. By clustering, we obtained 12 sets of core couplet herbs.

Conclusion: The syndrome differentiation of DN is not fixed but dynamic development progress. The syndromes and prescription regularities in the current 20 years are systematically summarized by data mining, which provides data support for clinical differentiation and further researches.

Key Words: Diabetic Nephropathy; Traditional Chinese medicine; syndromes distribution; Herbal medication rules

INTRODUCTION

Diabetic nephropathy (DN) is a widely recognized microvascular complication of diabetes and the largest single cause of the end-stage renal disease (ESRD), which is still a major contributor to morbidity and mortality of diabetic patients worldwide.¹⁻⁴ It is estimated that diabetes will reach 366 million by 2030, compared with more than 100 million for diabetic nephropathy.^{4,5} According to the data of American Diabetes Association, diabetes mellitus has a major impact on the development of DN. DN occurs in 20% to 40% of all patients with type 2 diabetes mellitus, accounting for approximately 50% of cases in the developed countries.⁶

Clinical manifestations of DN include an initial increase in glomerular filtration (GFR), proteinuria, increased creatinine levels, and eventually decreased GFR⁷. The major pathological alterations in DN include excessive accumulation and deposition of extracellular matrix, leading to expansion of mesangial matrix,

thickening of glomerular basement membrane and tubulointerstitial fibrosis.⁵ Based on the pathogenic factors of DN, a large number of novel treatment options have arisen from experimental studies, including intensive glycemic control, precise blood pressure control, optimal RAAS blockade with ACEI/ARB, lifestyle

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modifications such as exercise and dietary restrictions, and a lot of novel agents.^{1,8} In spite of the widespread application of numerous therapeutic approaches focusing on the management of factors mentioned above, the portion of ESRD due to DN still remains high. The interventions in general clinical use cannot efficiently slow or reverse the progression of nephropathy.⁷ Therefore, interventions that could optimally delay the development of DN are required.

Traditional Chinese medicine (TCM) has been widely used in the treatment of diabetes and its complications for a long time.⁹⁻¹¹ Up to now, there have been many experiments focusing on the treatment of diabetes and its complications with traditional Chinese medicine (TCM) because of less toxicity and/or side effects.^{12,13} From ancient times to the present, diabetic nephropathy has many diseases name in TCM, and it is equivalent to the category of "urine turbidity", "edema", "kidney fatigue", "Guan-ge" and "kidney-elimination" in TCM. According to traditional Chinese medicine, the basic pathogenesis of DN is the deficiency of both Qi and Yin, and the deficiency of both Qi and Yin throughout the development of DN. The main disease location is in the kidney, and is closely related to the liver, lung and spleen as well.¹⁰

To the present, little systematic research was done exploring the TCM syndrome distribution and herbal medication rules of DN. In this study, 274 prescriptions from the literature of TCM treatment of DN published in the last 20 years (1 January, 1998 - 30 June, 2019) were investigated, aiming to explore the TCM syndrome distributions and medication rules of TCM treatment for DN and shine valuable insight for TCM treatment of DN.

MATERIAL AND METHODS

Data sources

All the formulas were acquired from the literature of TCM treatment of DN published from 1 January, 1998 to 30 June, 2019 from both Chinese and English online databases. Set Traditional Chinese medicine or Chinese medicine or herbal medicine or herb or TCM or Chinese materia medica, and diabetic nephropathy or DN or diabetic kidney as the keyword, we searched the Chinese language databases consist of the China National Knowledge Infrastructure (CNKI) database, Wanfang database, the VIP Chinese Science and Technique Journals database. The English language databases we searched consist of PubMed, Embase, Web of Science and Cochrane Library.

Literature screening

Inclusion criteria:

1. Patients were clearly diagnosed as DN by Western medicine, the diagnosed criteria were reference to the statement declared by American Diabetes Association¹⁴.
2. TCM treatment was taken as a part of the overall DN treatment, and the treatment was effective reference to "Chinese and Western medicine diagnosis and treatment of diabetes and its complications" guideline.
3. TCM formula composition was clearly recorded.
4. Only oral formulas were included.

Exclusion criteria:

1. Literature with no exact diagnosis of DN by Western Medicine.
2. Formulas for external application.

Data standardization

The syndromes and pathological factors were standardized according to the National Standards of the People's Republic of China: *TCM clinical diagnosis and treatment terminology*.

Data entry

The selected prescriptions were entered into Excel 2007 manually by two researchers independently. After the entry was completed, the data was double-checked to ensure accuracy.

Software used

We used SPSS18.0 and SPSS Modeler14.1 for data analysis. The descriptive analysis and cluster analysis was performed by SPSS 18.0; the association rules analysis with the Apriori algorithm was performed by SPSS Modeler14.1.

RESULTS

After screening, 274 formulas were obtained. The meaningful results of frequency analysis, association rule analysis and cluster analysis were retained, and the repetitive and redundant rules were not retained.

Frequency analysis

According to the frequency, Table I shows the herbs with a frequency greater than or equal to 20; Table II shows the frequency of DN stages in literature Table III shows the frequency of the syndrome; Table IV frequency of the syndrome elements.

Frequency analysis of the herbs prescribed

274 prescriptions, including 213 herbs were obtained in this study; the total herb use frequency was 3015. We listed herbs with a frequency greater than or equal to 20 in Table I.

As it is shown in Table I, the most frequently used herbs are Milkvetch root (*Radix Astragali seu Hedysari*), Indian bread (*Poria*), Common yam rhizome (*Rhizoma Dioscoreae*), Danshen root (*Radix Salviae Miltiorrhizae*), Asiatic cornelian cherry fruit (*Fructus Corni*), Unprocessed Rehmannia root (*Radix Rehmanniae Recens*), Chinese angelica (*Radix Angelicae Sinensis*), Rhubarb root and rhizome (*Radix et Rhizoma Rhei*), Oriental water plantain rhizome (*Rhizoma Alismatis*), Largehead atractylodes rhizome (*Rhizoma Atractylodis Macrocephalae*), Sichuan lovage rhizome (*Rhizoma Ligustici Chuanxiong*), Prepared rehmannia root (*Radix Rehmanniae Preparata*), Tangshen (*Radix Codonopsis*), Tree peony root bark (*Cortex Moutan Radicis*), Heterophylly falsestarwort root (*Radix Pseudostellariae*).

Frequency of DN stages

Considering the inconsistency of staging diagnostic criteria for DN in the literature, the investigators re-delineated the medical records in each literature to "early-stage (I, II, III)", "medium stage (IV)" and "end-stage (V)" based on the Mogensen staging criteria¹⁵ and the TCM DN staging criteria put forward by Professor Lv Renhe¹⁶.

As it is shown in Table II, among the DN medical cases that treated by TCM, early-stage and medium stage take the most proportion, which indicates that TCM treatment is most commonly used in the early stage and medium stage of DN.

DN TCM syndrome distribution

Due to the complexity of DN pathogenesis, compound syndromes took a

large proportion in this study. We obtained 24 standard syndromes after classifying and standardizing the compound syndromes. Table III shows the syndromes with frequency greater than or equal to 10.

Table I Frequency of herbs used in the DN prescription

Herbs	Chinese	Function	Plant source	Frequency	Ratio
Milkvetch root (<i>Radix Astragali seu Hedysari</i>)	黄芪	①To stimulate the function of the spleen; ②to strengthen Qi	Astragalus membranaceus (Fisch.) Bge. var. mongholicus Bge. Hsiao.; Astragalus membranaceus (Fisch.) Bge.	209	76.28%
Indian bread (<i>Poria</i>)	茯苓	①To invigorate the spleen function; ②to cause diuresis	Poria cocos (Schw.) Wolf	122	44.53%
Common yam rhizome (<i>Rhizoma Dioscoreae</i>)	山药	①To benefit the stomach and spleen; ②to facilitate fluid secretion; ③to strengthen the kidney.	Dioscorea opposita Thunb.	119	43.43%
Danshen root (<i>Radix Salviae Miltiorrhizae</i>)	丹参	①To eliminate blood stasis; ②to stimulate the flow of blood; ③to relieve pain, and to ease the mind.	Salvia miltiorrhiza Bge.	108	39.42%
Asiatic cornelian cherry fruit (<i>Fructus Corni</i>)	山茱萸	①To complement the liver and kidney; ②to diminish collapse.	Cornus officie Sieb. et Zucc.	104	37.96%
Unprocessed rehmannia root (<i>Radix Rehmanniae Recens</i>)	生地	①To support yin and advance the production of body fluids; ②to clear heat and cool blood.	Rehmannia gultinosa Libosch.	88	32.12%
Chinese angelica (<i>Radix Angelicae Sinensis</i>)	当归	①To enrich the blood and activate blood circulation; ②to regulate menstruation, ③to lubricate bowels.	Angelica sinensis (Oliv.) Diels	70	25.55%
Rhubarb root and rhizome (<i>Radix et Rhizoma Rhei</i>)	大黄	①To cause catharsis and purge away heat; ②to reduce heat in the blood and counteract toxicity; ③to eliminate blood stasis and stimulate menstrual discharge.	Rheum palmatum L.; Rheum officinale Baill.; Rheum tanguticum Maxim. ex Balf.	69	25.18%
Oriental water plantain rhizome (<i>Rhizoma Alismatis</i>)	泽泻	①To influence diuresis; ②to eliminate damp-heat; ③to transform dampness and to stimulate water metabolism.	Alisma orientalis (Sam.) Juzep.	65	23.72%
Largehead atractylodes rhizome (<i>Rhizoma Atractylodis Macrocephalae</i>)	白术	①To strengthen the function of the spleen and benefit Qi; ②to remove damp; ③to strengthen diuretic function.	Atractylodes macrocephala Roidz.	62	22.63%
Sichuan lovage rhizome (<i>Rhizoma Ligustici Chuanxiong</i>)	川芎	①To invigorate blood and to promote Qi circulation; ②to expel wind and reduce pain	Ligusticum chuanxiong Hort.	61	22.26%
Prepared Rehmannia root (<i>Radix Rehmanniae Preparata</i>)	熟地	①To nourish Yin and replenish blood; ②to promote the essence of life and marrow.	Rehmannia gultinosa Libosch.	55	20.07%
Tangshen (<i>Radix Codonopsis</i>)	党参	①To promote Qi; ②to advance the function of the spleen.	Codonopsis pilosula (Franch.) Nannf.; Codonopsis pilosula Nannf. var. modesta (Nannf.) L. T. Shen; Codonopsis tangshen Oliv.	48	17.52%
Tree peony root bark (<i>Cortex Moutan Radicis</i>)	丹皮	①To clear heat from blood; ②to activate blood circulation and to remove blood stasis.	Paeonia suffruticosa Andr.	48	17.52%
Heterophylly falsestarwort root (<i>Radix Pseudostellariae</i>)	太子参	①To benefit Qi and active the spleen function; ②to reinforce fluid secretion.	Pseudostellaria heterophylla (Miq.) Pax ex Pax et Hoffm.	42	15.33%

Continued

Herbs	Chinese	Function	Plant source	Frequency	Ratio
Dodder seed (<i>Semen Cuscutae</i>)	菟丝子	①To arrest abnormal urination; ②to prevent abortion, ③to improve eyesight and relieve diarrhea by supplying and reinforcing the function of the liver and kidney.	<i>Cuscuta chinensis</i> Lam.; <i>Cuscuta japonica</i> Choisy	42	15.33%
Leech (<i>Hirudo</i>)	水蛭	To remove blood stasis	<i>Whitmania pigra</i> Whitman.; <i>Hirudo nipponica</i> Whitman.; <i>Whitmania acranulata</i> Whitman.	42	15.33%
Gordon euryale seed (<i>Semen Euryales</i>)	芡实	①To reinforce the kidney to retain the essence of life; ②to remove dampness and to strengthen the spleen; ③to alleviate loose bowels.	<i>Euryale ferox</i> Salisb.	39	14.23%
Cherokee rose fruit (<i>Fructus Rosae Laevigatae</i>)	金樱子	①To keep the essence of life; ②to advance the function of kidney; ③to stop diarrhea, to control urination.	<i>Rosa laevigata</i> Michx.	39	14.23%
Peony root (<i>Radix Paeoniae Rubra</i>)	赤芍	①To remove heat from blood; ②to remove blood stasis; ③to relieve pain.	<i>Paeonia lactiflora</i> pall.; <i>Paeonia veitchii</i> Lynch	38	13.87%
Barbary wolfberry fruit (<i>Fructus Lycii</i>)	枸杞	①To reinforce the kidney and liver and to supply the essence of life; ②to benefit eyesight.	<i>Lycium barbarum</i> L.	37	13.50%
Liquorice root (<i>Radix Glycyrrhizae</i>)	甘草	①To advance the function of the spleen and advance Qi; ②to dispel phlegm; ③to alleviate spasmodic pain; ④to moderate herbs and to decrease toxicity.	<i>Glycyrrhiza uralensis</i> Fisch; <i>Glycyrrhiza inflata</i> Bat.; <i>Glycyrrhiza glabra</i> L.	36	13.14%
Safflower (<i>Flos Carthami</i>)	红花	①To stimulate blood circulation; ②to eliminate blood stasis; ③to relieve pain.	<i>Carthamus tinctorius</i> L.	35	12.77%
Dwarf lilyturf tuber (<i>Radix Ophiopogonis</i>)	麦冬	①To nourish Yin and advance the production of body fluid; ②to benefit the lung; ③to ease the mind.	<i>Ophiopogon japonicus</i> (Thunb. Ker-Gawl.)	33	12.04%
Kudzu vine root (<i>Radix Puerariae</i>)	葛根	①To relieve fever; ②to stimulate the production of body fluid; ③to relieve diarrhea.	<i>Pueraria lobata</i> (Willd.) Ohwi; <i>Pueraria thomsonii</i> Benth.	32	11.68%
Motherwort herb (<i>Herba Leonuri</i>)	益母草	①To stimulate blood circulation and to eliminate blood stasis; ②to stimulate diuresis and relieve edema; ③to lower blood pressure.	<i>Leonurus heterophyllus</i> Sweet	32	11.68%
Peach seed (<i>Semen Persicae</i>)	桃仁	①To stimulate blood circulation; ②to remove phlegm; ③to smooth the bowels.	<i>Prunus persica</i> (L.) Batch.; <i>Prunus davidiana</i> (Carr.) Franch.	31	11.31%
Earthworm (<i>Lumbricus</i>)	地龙	①To remove heat; ②to remove obstruction from the collaterals; ③to advance diuresis and to relieve asthma; ④to remove blood stasis.	<i>Pheretima aspergillum</i> (E. Perrier); <i>Pheretima vulgaris</i> Chen; <i>Pheretima guillelmi</i> (Michaelsen); <i>Pheretima apectinifera</i> Michaelsen	30	10.95%
Figwort root (<i>Radix Scrophulariae</i>)	玄参	①To remove heat from blood; ②to advance Yin; ③to decrease toxicity.	<i>Scrophularia ningpoensis</i> Hemsl.	28	10.22%
Twotoothed achyranthes root (<i>Radix Achyranthis Bidentatae</i>)	怀牛膝	①To supply the liver and the kidney; ②to strengthen the bones; ③to remove blood stasis; ④to lead the blood to flow downward.	<i>Achyranthes bidentata</i> Bl	28	10.22%
Solomonseal rhizome (<i>Rhizoma Polygonati</i>)	黄精	①To benefit Qi and Yin; ②to advance the function of the spleen; ③to benefit the kidney.	<i>Polygonatum kingianum</i> Coll. et Hemsl.; <i>Polygonatum sibiricum</i> Red.; <i>Polygonatum cyrtoneura</i> Hua	28	10.22%
Epimedium herb (<i>Herba Epimedii</i>)	仙灵脾	①To promote the Yang of the kidney; ②to advance the tendons and bones; ③to relieve rheumatic disease.	<i>Epimedium brevicornum</i> Maxim.; <i>Epimedium sagittatum</i> (Sieb. et Zucc.) Maxim.; <i>Epimedium pubescens</i> Maxm.; <i>Epimedium wushanense</i> T. S. Ying; <i>Epimedium koreanum</i> Nakai	27	9.85%

Continued

Herbs	Chinese	Function	Plant source	Frequency	Ratio
Golden thread (<i>Rhizoma Coptidis</i>)	黄连	①To eliminate damp-heat and to reduce the fire; ②to counteract toxicity.	Coptis Chinensis Franch.; Coptis deltoidea C. Y. Cheng et Hsiao; Coptis teeta Wall.	26	9.49%
Sanqi (<i>Radix Notoginseng</i>)	三七	①To remove blood stasis; ②to prevent bleeding and to relieve pain.	Panax notoginseng (Burk.) F. H. Chen.	26	9.49%
Glossy privet fruit (<i>Fructus Ligustri Lucidi</i>)	女贞子	①To advance the function of the liver and kidney;②to benefit eyesight and to benefit the growth of hair.	Ligustru lucidu Ait.	26	9.49%
Chinese magnoliavine fruit (<i>Fructus Schisandrae Chinensis</i>)	五味子	①To arrest discharges; ②to advance Qi and to stimulate the fluid secretion;③to reinforce the kidney.	Schisandra chinensis (Turcz.) Baill.; Schizandra spheanthera Rehd. Et Wils.	25	9.12%
Winged Euonymus Twig (<i>Ramulus Euonymi</i>)	鬼箭羽	①To activate blood and to remove blood stasis; ②to relief pain	Euonymus alatus(Thunb.) Sieb	25	9.12%
Prepared common monkshood branched root (<i>Radix Aconiti Lateralis Preparata</i>)	附子	①To promote Yang and to advance the function of kidney;②to cause restoration from collapse; ③to dispel wind, cold and damp.	Aconitum carmichaeli Debx.	24	8.76%
Debark peony root (<i>Radix Paeoniae Alba</i>)	白芍	①To soothe liver Yang; ②to supply blood and nourish the Yin; ③to relief pain.	Paeonia lactiflora pall.	20	7.30%

Table II Frequency of DN stage

DN Stage	Frequency	Ratio
Early-stage	147	53.65%
Medium stage	96	35.04%
End-stage	31	11.31%

Table III Frequency of DN syndrome

Syndrome	Frequency	Ratio
Deficiency Qi and Yin	112	40.88%
Obstruction of collaterals by blood stasis	78	28.47%
Deficiency of liver and kidney	62	22.63%
Damp-heat stasis obstruction	48	17.52%
Yin asthenia generating intrinsic heat	42	15.33%
Phlegm and blood stasis	35	12.77%
Deficiency of yin and dampness-heat	30	10.95%
The mutual knot of damp heat and phlegm and stasis	27	9.85%
Hyperactivity of fire due to deficiency of kidney	24	8.76%
Asthenia of both the spleen and kidney	19	6.93%
The weakness of the spleen and the stomach	17	6.20%
Qi stagnation and blood stasis	14	5.11%
Deficiency of spleen-yang and kidney-yang	12	4.38%
Wind formation from blood dryness	11	4.01%

As it is shown in Table III and Figure 1, the most common syndromes of DN are deficiency Qi and Yin, obstruction of collaterals by blood stasis, deficiency of liver and kidney,

damp-heat stasis obstruction, Yin asthenia generating intrinsic heat, and Phlegm and blood stasis.

Figure 1 frequency of DN syndrome

Frequency of syndrome pathological element

The concept of syndrome element was put forward by Professor Zhu Wenfeng^{17,18}, which had an important influence on TCM academic development. The syndrome element is the smallest unit of TCM syndromes. As it is shown in Table IV and Figure 2, the most frequently common syndrome elements of DN are Yin deficiency, blood stasis, Qi deficiency, endogenous heat and phlegm.

3.2 Association rules analysis

To explore the association rules among herbs used in DN, we

Table IV Frequency of syndrome elements

Syndrome elements	Frequency	Ratio
Yin deficiency	194	70.80%
Blood stasis	168	61.31%
Qi deficiency	159	58.03%
Endogenous heat	142	51.82%
Phlegm	126	45.99%
Turbid bane;	75	27.37%
Kidney deficiency	85	31.02%
Yang deficiency	62	22.63%
Blood deficiency	49	17.88%
Dryness	38	13.87%

perform association analysis by the Apriori algorithm. The association rule was ranked by confidence. Confidence stands for the percentage of rule support to antecedent support; while support stands for the probability of the antecedent and consequent appearing at the same time. Only confidence greater than 85% and support greater than 15% were displayed in Table V. In Figure 3, the thickness of the line represents the degree of association, the thicker

the line, the higher the degree of association.

3.3 Cluster analysis

To explore the herbal medication rules in TCM treatment of DN, we perform cluster analysis on herbs with frequency more than or equal to 30 were analyzed by cluster analysis. Basic information about the core herbs obtained from the results of cluster analysis is presented in Figure 4 and Figure 5.

Figure 2 Frequency of syndrome elements **Figure 3** Association results of herbs used in DN**Table V** Herb association rules

Consequent	Antecedent	Support %	Confidence %
Milkvet root = T	Asiatic cornelian cherry fruit = T and Danshen root = T	18.02	95.92
Milkvet root = T	Danshen root = T and Indian bread = T	17.28	95.75
Milkvet root = T	Danshen root = T and Common yam rhizome = T	23.53	95.31
Milkvet root = T	Asiatic cornelian cherry fruit = T and Danshen root = T and Common yam rhizome = T	15.07	95.12
Milkvet root = T	Sichuan lovage rhizome = T	22.43	95.08
Milkvet root = T	Tangshen = T	17.65	93.75
Milkvet root = T	Danshen root = T	39.71	89.81
Milkvet root = T	Asiatic cornelian cherry fruit = T and Common yam rhizome = T	27.94	89.47
Milkvet root = T	Indian bread = T and Common yam rhizome = T	26.84	89.04
Milkvet root = T	Rhubarb root and rhizome = T	25.37	88.41
Milkvet root = T	Common yam rhizome = T	43.75	88.24
Milkvet root = T	Asiatic cornelian cherry fruit = T and Indian bread = T and Common yam rhizome = T	18.75	88.24
Milkvet root = T	Chinese angelica = T	25.74	87.14
Common yam rhizome = T	Asiatic cornelian cherry fruit = T and Indian bread = T and Milkvet root = T	19.12	86.54
Milkvet root = T	Unprocessed rehmannia root = T	32.35	86.36
Common yam rhizome = T	Oriental waterplantain rhizome = T and Asiatic cornelian cherry fruit = T	15.44	85.71
Asiatic cornelian cherry fruit = T	Oriental waterplantain rhizome = T and Common yam rhizome = T	15.44	85.71
Indian bread = T	Oriental waterplantain rhizome = T and Common yam rhizome = T	15.44	85.71
Milkvet root = T	Asiatic cornelian cherry fruit = T	38.24	85.62
Common yam rhizome = T	Prepared rehmannia root = T	44.85	85.43

Figure 4 Icicle chart of herbs (with frequency more than or equal to 30) used in DN

Figure 5 Dendrogram of herbs (with frequency more than or equal to 30) used in DN

By clustering, 12 sets of core couplet medicines were obtained: 1. Unprocessed Rehmannia root & Asiatic cornelian cherry fruit & common yam rhizome; 2. Tree peony root bark & prepared Rehmannia root; 3. Chinese angelica & tree peony root bark & prepared Rehmannia root; 4. Tangshen & largehead atractylodes rhizome; 5. Sichuan lovage rhizome & oriental water plantain rhizome; 6. Twotoothed achyranthes root & motherwort herb; 7. Heterophylly falsestarwort root & figwort root & dwarf lilyturf tuber; 8. Figwort root & dwarf lilyturf tuber; 9. Peach seed & safflower; 10. Peony root & earthworm & peach seed & safflower; 11. Barbary wolfberry fruit & dodder seed; 12. Cherokee rose fruit & Gordon euryale seed.

DISCUSSION

Overall, in our study, by analyzing 274 prescriptions used in TCM for DN in the recent 20 years, we found that DN is quite complicated and is a combination of deficiency and excess in the TCM perspective, which is consistent with other views from ancient times to the present. The results of the paper suggest that the use of herbs is steady with the DN pathogenesis. Syndrome differentiation, proposed by the TCM Saint Zhang Zhongjing (circa 150-154 A.D. to 215-219 A.D.), is the basis in TCM treatment, which means personalized treatment based on different patients and different TCM syndrome.¹⁹ Therefore, the study of syndromes plays a key role in the TCM treatment of DN.²⁰ According to the guidelines for prevention and TCM treatment of DN proposed by the Chinese Medicine Association, the pathogenesis of diabetic nephropathy is based on the deficiency of Qi and Yin. So tonify Qi and Yin go throughout the treatment.⁶ Our study supports this view. In our study, the five most common syndromes of DN are Yin deficiency, blood stasis, Qi deficiency, endogenous heat and phlegm. Within them, the frequency of deficiency Qi and Yin is 112, and the ratio is 40.88%, accounting for the highest proportion. The frequency of obstruction of collaterals by blood stasis was 78, and the percentage was 28.47%. The frequency of deficiency of liver and kidney was 62, and the ratio was 22.63%. Clearly, these three syndromes accounted for the largest proportion. In addition, the frequency of damp-heat stasis obstruction was 48, and the ratio was 17.52%. The frequency of Yin asthenia generating intrinsic heat was 42, and the percentage was 15.33%. To be more specific, we further explored the pathological elements of DN, which shows that the five most general pathological elements are Yin deficiency (70.80%), blood stasis (61.31%), Qi deficiency (58.03%), endogenous heat (51.82%) and phlegm (45.99%).

Although there is a common consensus that the basis of Qi and Yin deficiency is the basis of DN, due to the complex and compound syndrome differentiation system in TCM, debates on the syndrome of DN never stop.²¹⁻²⁴ This study shows that the basic pathogenesis of DN is the deficiency of Qi and Yin, while blood stasis runs through the whole course of DN based on the saying, "long-term chronic diseases transforming to collaterals." As it is fully understood that the syndrome of DN is not fixed but a dynamic development progress.²⁵ In TCM perspective, in the early stage of the disease, the lung, stomach, lung and kidney suffer from the loss of Yin fluid. Throughout time, the Yin deficiency affects Qi and Yang, causing Qi and Yin deficiency, Yin and Yang deficiency, and spleen and kidney Yang deficiency.²⁰ Throughout the disease, intermingled deficiency and excess occur, such as Qi and Yin deficiency with fewer blood veins astringent, Qi and blood

deficiency with cold stagnation and blood stasis, etc., while dampness and blood stasis are the important factors cannot be ignored. With the progression of DN, the clinical manifestations of water dampness and blood stasis are gradually aggravated. Water dampness repression and stagnation of blood stasis are not only related to the activity of the disease, but also an important factor leading to aggravating the impairment of renal function.²⁴

In addition to syndromes, herbs are important carriers of TCM. As we know, inflammation and oxidative stress are two closely linked DN processes.²⁶⁻²⁸ Current research shows that lots of herbs have been proven to have anti-inflammatory and antioxidative effects, such as Milkvetch root,²⁹ Danshen root,³⁰ and Largehead atractylodes rhizome,³¹ etc. In addition, renal fibrosis is the ultimate common pathway leading to the early development of chronic kidney disease progress to end-stage renal disease. Therefore, anti-fibrotic therapy is an effective treatment for DN. A lot of herbs such as Chinese angelica, Milkvetch root, Rhubarb root and rhizome, are proven to have antifibrotic effect and immunomodulation effects as well.³²⁻³⁵ To investigate the most frequently used herbs in DN, we rank the herbs in terms of the frequency. Our analysis results suggest that herbs with number of times greater than 30 are as follows: milkvetch root, Indian bread, common yam rhizome, danshen root, Asiatic cornelian cherry fruit, unprocessed Rehmannia root, Chinese angelica, rhubarb root and rhizome, oriental water plantain rhizome, largehead atractylodes rhizome, Sichuan lovage rhizome, prepared Rehmannia root, Tangshen, tree peony root bark, heterophylly falsestarwort root, dodder seed, leech, gordon euryale seed, cherokee rose fruit, peony root, barbary wolfberry fruit, liquorice root, safflower, dwarf lilyturf tuber, kudzu vine root, motherwort herb and peach seed. The frequency of Milkvetch root is 209 and 76.28% of prescriptions collected contain it.

Milkvetch root is used to fortify Qi, the vital energy of life. It promotes the protective Qi and solidifies the external of the body as well. In TCM theory, it is sweet and slightly warm in nature. It belongs to the meridian of the spleen and lungs. It is reported to ① raise the sum of polymorphocytes and leukocytes in peripheral blood and strengthening the lymphocyte-blastogenesis; ② cardiotonic; ③ dilate coronary artery and capillaries.²⁹ Danshen root, Chinese angelica, rhubarb root and rhizome, Sichuan lovage rhizome, leech and safflower can stimulate blood circulation and eliminate blood stasis. Danshen root can dispel the blood stasis and relief pain, stimulate the blood and remove heat. It is reported that ① the tanshinone can be used as a broad-spectrum bactericide, can stimulate the coronary flow, and expand coronary artery; ② influence many blood clotting factors, control the aggregation of platelet and improve the level of cAMP in thrombocytes.³⁰ Chinese angelica can improve the blood, relief pain and stimulate blood circulation. It is reported to ① diminish myocardial ischemia actuated by pituitary; ② expand coronary artery, reduce oxygen consumption and improve coronary flow.³⁶ Rhubarb is usually used to decrease heat and moves the bowels, clears heat from the blood, dispels stasis and unblocks the Channels. It can strongly dispel blood stasis and reduces immobile masses. It is reported to ① Emodin carry out bacteriostatic influence through restraining the synthesis of RNA, DNA and protein in bacteria; ② Emodin plays role as a laxative, a tyrosine kinase inhibitor, an antineoplastic agent, and a plant metabolite; ③ facilitate the biliary secretion and promote the sum of bile acid and bilirubin.³⁷ Safflower promotes the blood, disperses stasis, relief pain, and unblocks the Channels. It is reported to ① improve the coronary

flow; ② stimulates uteri and intestines in vitro; ③ lower blood pressure.³⁸ Tree peony root bark can activate blood circulation, clear pathogenic heat from the blood, and remove blood stasis. It is reported to ① antibacterial and anti-inflammatory; ② analgesic.³⁹ Unprocessed Rehmannia root can be used to cool blood, remove heat, nourish yin, and stimulate the production of fluids. It is reported to lower the blood sugar level.⁴⁰ Heterophyllally falsestarwort root can strengthen Qi, advance the spleen function, and stimulate fluid secretion.⁴¹ Indian bread and largehead atractylodes rhizome can clear damp, promote diuresis, strengthen the spleen and reduce phlegm. Indian bread is commonly used to ① treat edema with oliguria; ② diminished function of the spleen, which is marked by anorexia dizziness; ③ palpitation result in retained fluid.⁴² Largehead atractylodes rhizome is usually used to eliminate damp by causing diuresis, to promote the spleen, to replenish qi, and is proven to promote phagocytosis and the function of adrenal cortex⁴³. Common yam rhizome advances the function of the spleen and stomach, promotes the function of the kidneys, and generates fluids. It is proven to promote the phagocytosis of leukocyte in vitro.⁴⁴ To conclude, herbs for tonifying Qi, nourishing Yin, stimulating blood circulation to remove blood stasis, nourishing liver and kidney, removing phlegm take account for a large proportion among the high-frequency medicinal to treat DN.

In addition, the accumulated experiences in the practice of TCM indicate that the combination of herbs is more effective than herb used in isolation. So we not only emphasize the isolated herbs but also the combined herbs in prescriptions. In accordance with the data mining analyses result, 13 sets of commonly-used combined herbs are listed and discussed here.

1) Rehmannia root & Usiatic cornelian cherry fruit & Common yam rhizome. Rehmannia root, usiatic cornelian cherry fruit and common yam rhizome are combined herbs in Liuwei Dihuang Wan, which are commonly used to enrich the kidneys Yin. Rehmannia root can remove heat and cool the blood, enhance Yin and generates fluids; usiatic cornelian cherry fruit can benefit the liver and kidneys, and secures conditions from collapsing; common yam rhizome nourishes the spleen and stomach, generates fluids, advances the function of the kidneys and retains Jing.

2) Chinese angelica & Tree peony root bark & prepared Rehmannia root. Chinese angelica is sweet, acrid and warm in nature, and it belongs to Channel of liver, heart and spleen. Chinese angelica nourishes and invigorates the blood, regulates menses and relieves pain, and moistens the intestines and moves the bowels. As is mentioned above, tree peony root bark and prepared Rehmannia root can both tonify Yin and kidney, and clear heat and invigorate the blood. With Chinese angelica, this herb combination can nourish the blood, tonify Yin and kidney, and clear heat and invigorate the blood.

3) Peony root & earthworm & Peach seed & safflower. This combination is commonly used to invigorate the blood. Tree peony root bark clears heat and cools the blood and invigorates the blood and dispels the blood stasis. Clears heat and settles fright, unblocks the collaterals, calms wheezing and promotes urination. Peach seed invigorates the blood and dispels blood stasis, and moistens the intestines and moves the bowels. Safflower invigorates the blood and unblocks the channels and disperses stasis and alleviates pain.

4) Barbary wolfberry fruit & dodder seed. Barbary wolfberry fruit nourishes and tonifies the liver and kidneys and benefits Jing and improves vision. Dodder seed tonifies the liver and kidneys

and secures Jing and reserves urine. The compatibility of barbary wolfberry fruit and dodder seed can increase strength of tonifying the liver and kidneys and securing Jing and reserving urine.

5) Cherokee rose fruit & Gordon Euryale seed. Cherokee rose fruit secures Jing and contains urination and binds up the intestines and stops diarrhea. Gordon Euryale seed strengthens the spleen and stops diarrhea, stabilizes the kidneys and astringes Jing, expels dampness and stops the discharge. The compatibility of Cherokee rose fruit and Gordon euryale seed strengthens the spleen and kidney, and expels dampness and stops discharge.

6) Tree peony root bark & prepared Rehmannia root. Tree peony root bark influences heart, liver and kidney, and can clear heat and cools the blood and invigorates the blood and dispels the blood stasis. Prepared Rehmannia root influences liver, kidney, and can tonify Yin and nourish the blood and tonify Jing and supplement bone marrow. This herb combination can both tonify Yin and kidney, and clear heat and invigorate the blood.

7) Sichuan lovage rhizome & oriental water plantain rhizome. Oriental water plantain rhizome is sweet and cold in nature, and it belongs to the channel of kidneys and bladder. Oriental water plantain rhizome promotes urination and drains damp-heat. Sichuan lovage rhizome invigorates the blood and moves Qi and expels wind and alleviates pain. The compatibility of oriental water plantain rhizome and Sichuan lovage rhizome can both promote blood and Qi circulation, and drain damp-heat.

8) Milkvetch root & Lovage rhizome. Milkvetch root is warm and sweet in nature, and it belongs to the channel of lung and spleen. It is usually used to reinforce Qi and invigorates the function of the spleen. Lovage rhizome is pungent and bitter in nature, and belongs to channel of bladder and liver. It can be used to relieve rheumatic conditions and to remove obstruction from the channels and relieve pain. The combined herbs can not only reinforce Qi but also remove obstruction from the channels and relieve pain.

9) Twotoothed achyranthes root & motherwort herb. Twotoothed achyranthes root tonifies the liver and kidneys, strengthens sinews and bones, dispels stasis and unblock the channels, induce the blood moving downward. Motherwort herb invigorates the blood and regulates menses and promotes urination and reduces swelling. The compatibility of two toothed achyranthes root and motherwort herb can both tonify the liver and kidneys and invigorate the blood.

10) Heterophyllally falsestarwort root & Figwort root & dwarf lilyturf tuber. Heterophyllally falsestarwort root tonifies Qi and strengthens the spleen, and generates fluids and moistens the lungs. Figwort root cools the blood and nourishes Yin, and purges fire and resolves toxicity. Dwarf lilyturf tuber nourishes Yin and moistens lungs, and clears heart and calms the spirit. The compatibility is a common combination to nourish Yin, clear heart and notify Qi and strengthens the spleen.

11) Figwort root & dwarf lilyturf tuber. Figwort root cools the blood and nourishes Yin, and purges fire and resolves toxicity. Dwarf lilyturf tuber nourishes Yin and moistens the lungs, and clears heart and calms the spirit. The compatibility of figwort root and dwarf lilyturf tuber is a common combination to nourish Yin and clear heart.

12) Peach seed & safflower. Peach seed invigorates the blood and dispels blood stasis, and moistens the intestines and moves the bowels. Safflower invigorates the blood and unblocks the channels and disperses stasis and alleviates pain. Two herbs used together can promote each other, and increase the strength of promoting

blood circulation, removing new blood stasis, swelling and pain relief.

13) Tangshen & largehead atractylodes rhizome. Tangshen is sweet and neutral in nature, and it tonifies the middle burner and augments Qi, strengthens the spleen and benefits the lungs. Largehead atractylodes rhizome is acrid, bitter and warm in nature, and belongs to the meridian of spleen, stomach and liver. Largehead atractylodes rhizome dries dampness and strengthens the spleen, expels wind and disperses cold. This herb combination can both tonify the middle burner and augments Qi, and dries dampness and strengthens the spleen.

CONCLUSION

The syndrome differentiation of DN is not fixed but dynamic development progress. In TCM theory, DN is quite complicated and is a combination of deficiency and excess. As we know, herbs are important carriers of TCM. Although lots of herbs are proven to have anti-inflammatory, antioxidative and anti-fibrotic effects, the usage is complicated as well. TCM has certain advantages in the prevention and treatment of DN, which is affirmed by most TCM medical practitioners, but there is no uniform standard for syndrome differentiation and efficacy evaluation. TCM treatment of DN is better in the early and middle stages, especially in the early stage. In the middle and late stages of DN, single Chinese medicine cannot alleviate the condition and requires combination therapy of Western medicine. At present, the mechanism of prevention and treatment of DN by TCM is still unclear. The evidence-based medical studies for DN are limited and there is a lack of the multi-center large-scale clinical research as well.

TCM is more than "Artemisinin". We would better abandon prejudice and understand the advantages and disadvantages of both Chinese and Western medicine in the treatment of DN. We fully expect that the results of this research could shine valuable insight into TCM treatment of DN.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts

The authors declare no conflicts of interest.

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