ORIGINAL RESEARCH

Corneal Specular Microscopy Findings in Patients of Type 2 Diabetes Mellitus at Tertiary Care Center

[1]Lakshita Maherda, [2]Kalpna Jain[2], [3]Poonam Bhargava, [4]Sajjan Singh Meena

[1,4]Resident, [2]Senior Professor & HOD, [3]Associate Professor, Department of Ophthalmology, S. P. Medical College, Bikaner, Rajasthan, India

Correspondence:
Lakshita Maherda
Resident, Department of Ophthalmology, S. P. Medical College, Bikaner, Rajasthan, India
Email: maherdalakshita@gmail.com

ABSTRACT
Background: Diabetes mellitus has been postulated to alter the morphology of the cornea.Aim of our study was to compare the morphological characteristics of corneal endothelial cellsin type 2 diabetic patients with age-matched healthy subjects. We also determined the association of corneal morphological features with the general characteristics and laboratory data of diabetic patients, including disease duration, urine albumin creatinine ratio.
Methods:A case-control study in a tertiary care hospital of northwestern India with a total of 50 diabetic patients and 50 healthy controls were enrolled in the study. All participants underwent a complete ophthalmological examination. Corneal endothelial measurements were performed using a noncontact specular microscopy. Laboratory data including serum fasting glucose, --HbA1c levels, creatinine levels, and the urinary albumin-to-creatinine ratio were recorded. Diabetic patients were further subdivided into 3 groups according to the presence and stage of diabetic retinopathy. Specular microscopy findings and central corneal thickness of all patients were compared.
Results: The ECD and hexagonal cell ratio were significantly lower, while the average cell size, CV%, and central corneal thickness were determined to be significantly higher in diabetic patients than in healthy controls (p = 0.001). With the presence and advancement of diabetic retinopathy, the ECD and hexagonal cell ratio decreased, while the average cell size, CV%, and central corneal thickness increased. When correlation analysis was performed between corneal morphological features and laboratory data of diabetic patients, ECD showed a significant negative correlation with diabetes duration (p = 0.028). Urinary albumin-creatinine ratio (p = 0.041), average cell size and CV showed a positive correlation with these parameters.
Conclusion: In conclusion, keratopathy is an important complication of type 2 diabetes. With an increase in the stage of diabetic retinopathy, alterations in corneal findings also increased. In that respect, we can suggest that keratopathy should be evaluated more cautiously in diabetic patients.
Keywords: Specular Microscopy, Corneal Endothelial Cells, Diabetes Mellitus, Microalbuminuria.

BACKGROUND

Diabetes mellitus is considered a major non-communicable disease worldwide. Based on projections made by International Diabetes Federation (IDF), 80 % of new cases are expected to occur in the developing world and in India diabetic population is expected to double by

year 2030.[1,2]Diabetes mellitus (DM) is associated with damage and dysfunction of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[3] Diabetic retinopathy (DR) is one of the most important causes of blindness and affects approximately 40% of all diabetic patients.[4]

In general, diabetes affects both vascular and neural cells of the retina and is characterized by alterations of the retinal microvasculature.[5,6]However, functional abnormalities have been documented in cornea too like decreased endothelial cell density (ECD), increased corneal thickness and corneal keratopathy.[7-12] Corneal endothelial cell is responsible for maintaining the transparency of the cornea.[8] There is limited ability of mitosis in corneal endothelium and once damaged, remaining cells enlarge to cover up the lost area.[13]There will be an increase in variation of cell area called polymegathism or coefficient of variation (CV) and index of hexagonality (6A) or pleomporphism.[14]

Central corneal thickness (CCT) can be used as a marker of endothelial health and can be used to monitor corneal edema. There is a postulated association between corneal thickness and severity of diabetic retinopathy.[15-17] Age can always be considered as a confounding factor in studying disease of corneal endothelium and endothelial cell density has been found to decrease with age.[18,19]

Specular microscopes used today are based on design suggested by Maurice., et al.[20] The modern noncontact specular microscope to study corneal endothelium employ automated interfacing for obtaining image through a discrete focusing technology.[21]Considering the huge diabetic population in India and paucity of literature especially in India, a study was proposed to evaluate corneal endothelial changes using specular microscope in patients of type 2 diabetic mellitus.

Our study aimed to compare the morphological characteristics of corneal endothelial cells in type 2 diabetic patients and age-matched healthy subjects by specular microscopy. We also aimed to analyze alterations in the cornea in diabetic patients with advanced-stage diabetic retinopathy. Moreover, we aimed to determine the association of corneal morphological features with the general characteristics and laboratory data of diabetic patients, including disease duration.

METHODS

This prospective study was performed at Sardar Patel Medical College and PBM Hospital, Bikaner between 01/2021 and 07/2021. The study was approved by the local ethics committee, and informed consent was obtained from all participants. In total,50 diabetic patients and 50healthy, control participantswere concomitantly enrolled in the study. The exclusion criteria were the use of contact lenses; presence of dry eye disease; history of any ocular diseases including corneal scar, cataract or glaucoma; and any history of previous ocular surgery.

All participants underwent a complete ophthalmic evaluation, including Slit-lamp biomicroscopic examination, Goldman applanation tonometry, and Fundoscopy with pupil dilation. Grading of DR was based on the International Clinical Diabetic Retinopathy Disease Severity Scale as without DR, with background DR or with proliferative DR.[22] The patients who were diagnosed with type 2 diabetes and under follow-up in our hospital were included in the study. The diagnosis of diabetes was based on the ADA (American Diabetes Association) criteria; fasting blood glucose ≥126 mg/dl on two separate occasions, random blood glucose (RBG) ≥200 mg/dl with symptoms or 2-h plasma glucose ≥200 mg/dl.[23]

Diabetic patients were further subdivided into 3 groups based on the presence and stage of diabetic retinopathy as patients without DR, with background DR, and with proliferative DR After 8 h of fasting, serum samples of study participants were obtained to analyze serum glucose.Both eyes of all participants were examined. Endothelial cell density (ECD), average

cell size, percentage of hexagonal cells, coefficient of variation (CV) in cell size, and central corneal thickness (CCT) of all patients were measured using a noncontact specular microscopy (CEM-530 Specular Microscope, NIDEK, Japan) device. All measurements were performed in the morning between 9:00 and 11.00 am by the same skilled ophthalmologist.

STATISTICAL ANALYSIS

Data analysis was performed using SPSS software (version 21.0, SPSS, Inc.). The normality of the distribution of the data was analyzed with the Shapiro–Wilk test, and since all data were normally distributed, parametric tests were used. For the comparison of two groups, independent samples t-test and chi-square test were performed. Pearson correlation analysis was used to examine the correlation of corneal findings with the clinical characteristics of diabetic patients, including disease duration.

RESULTS

In total, 100 eyes of 50 patients (21 male, 29 female) with type 2 diabetes mellitus were included in the study. In the control group, 100 eyes of 50 (23 male, 27 female) healthy cases with normal fasting blood glucose. There was no significant difference between groups regarding sex (p = 0.06) or age (p = 0.135) (Table 1). Fasting blood glucose levels, HbA1c and urinary albumin-creatinine ratio levelswere significantly higher in the diabetic group (p = 0.001). The ECD and hexagonal cell ratio were significantly lower, while the average cell size, CV%, and CCT were determined to be significantly higher in diabetic patients than in healthy controls (Table 2). Demographic features, ocular findings and laboratory data of diabetic patients are summarized in Table 3. There was no significant difference between groups regarding sex or age. With the presence and advancement of DR, the ECD and hexagonal cell ratio decreased, while the average cell size, CV%, and CCT increased (Table 3) Diabetic patients with (n:50) or without (n:50) microalbuminuria were compared in regard to demographic features and corneal findings and the data are summarized in Table 4.

There was no significant difference between groups regarding sex or age. In diabetic patients with microalbuminuria, ECD was significantly lower (2325.25 ± 147.45 vs. 2458.44 ± 183.92 cells/m2; p = 0.004), while average cell size (432.44 ± 37.80 vs. 404.80 ± 76.98; p = 0.038) and CV% (32.98 ± 5.72 vs 31.35 ± 6.51; p = 0.004) were significantly higher.

When correlation analysis was performed between the corneal morphological features and general characteristics and laboratory data of diabetic patients, ECD showed a significant negative correlation with diabetes duration (p = 0.028) and urinary albumin-creatinine ratio (p = 0.041), and average cell size and CV showed a positive correlation with these parameters (Table 5).

Table 1: Demographic features of study participants

	Diabetic group (n: 50)	Healthy controls (n: 50)	P
Gender (M/F)	21/29	23/27	0.06
Age (years)	62.256±8.213	58.945±7.584	0.135

Table 2: Comparison of laboratory data and ocular findings of study groups

	Diabetic group (n: 50)	Healthy controls (n: 50)	P
Fasting Blood Glucose (mg/dl)	176.06 ± 55.52	94.37 ± 11.08	0.001
HbA1c(%)	7.54±1.01	5.24±0.58	0.001
Blood urea nitrogen	37.77 ± 12.94	5.26 ± 0.72	0.001
Creatinine	0.98 ± 0.32	30.11 ± 6.88	0.001
Urine albumin-creatinine ratio (mg/gCre)	54.3473 ± 59.82	0.81 ± 0.27	0.001

Endothelial Cell Density (cells/m2)	2427.94 ± 427.10	0.81 ± 0.27	0.001
Average cell size (µm2)	419.69 ± 71.98	374.74 ± 14.11	0.001
Coefficient of Variation (%)	32.37 ± 6.16	30.29 ± 4.08	0.001
Hexagonal cell ratio (%)	66.94 ± 7.17	78.48 ± 6.56	0.001
Central corneal thickness (µ)	539.01 ± 41.19	525.90 ± 28.79	0.001

Table 3: Demographic features, laboratory data and ocular findings of three subgroups of diabetic patients

	Without DR (n: 30)	Background DR (n: 28)	Proliferative DR (n: 32)	P1	P2	P3
Gender (M/F)	8/10	6/8	8/10	0.52	0.14	0.25
Age (years)	61.52 ±7.459	63.45 ± 8.25	62.58 ± 7.37	0.144	0.128	0.257
Diabetes duration (years)	5.30 ± 1.96	9.32 ± 3.52	13.05 ± 4.11	**0.001**	**0.001**	**0.001**
Fasting Blood Glucose (mg/dl)	138.23 ±35.40	190.32 ± 51.16	205.25 ± 56.72	**0.001**	**0.001**	**0.001**
Blood urea nitrogen	30.62 ± 9.04	39.11 ± 11.48	44.42 ± 12.87	**0.001**	**0.001**	**0.001**
Creatinine	0.82 ± 0.25	1.03 ± 0.29	1.15 ± 0.25	**0.001**	**0.001**	**0.001**
Urine albumin-creatinine ratio (mg/g·Cre)	9.80 ± 4.84	52.27 ± 41.42	120.30 ± 62.98	**0.001**	**0.001**	**0.001**
Endothelial Cell Density (cells/m^2)	2455.43 ± 362.30	2372.18 ± 460.70	2395.26 ± 431.90	**0.041**	**0.020**	0.871
Average cell size (µm^2)	401.42 ± 63.32	437.42 ± 76.18	446.36 ± 63.05	**0.012**	**0.008**	0.304
Coefficient of Variation (%)	31.54 ± 4.89	31.54 ± 5.26	33.38 ± 5.28	**0.042**	**0.004**	0.408
Hexagonal cell ratio (%)	64.18 ± 6.40	64.50 ± 5.60	67.58 ± 7.54	0.436	0.410	0.112
Central corneal thickness (µ)	523.29 ± 52.62	525.54 ± 33.42	552.45 ± 33.05	0.467	0.278	0.654

M male, F female; p1 p value of the comparison between Without DR and Background DR groups; p2 p value of the comparison between Without DR and Proliferative DR groups; p3 p value of the comparison between Background DR and Proliferative DR groups

Table 4: Comparison of corneal findings in diabetic patients with or without microalbuminuria

	Diabetic patients with microalbuminuria(n:26)	Diabetic patients without microalbuminuria (n: 24)	P
Gender (M/F)	11/15	11/13	0.24
Age (years)	61.54 ± 8.56	63.41 ± 7.11	0.11
Diabetes duration (years)	11.12 ± 4.07	6.56 ± 3.53	0.001
Endothelial Cell Density (cells/m^2)	2325.25 ± 147.45	2458.44 ± 178.82	0.004
Average cell size (µm^2)	432.44 ± 37.80	404.80 ± 76.98	0.038
Coefficient of Variation	32.98 ± 5.72	31.35 ± 6.51	0.004

(%)			
Hexagonal cell ratio (%)	65.85 ± 5.45	67.14 ± 8.27	0.293
Central corneal thickness (μ)	539.19 ± 32.84	537.83 ± 47.46	0.891

Table 5: Results of correlation analysis performed between corneal morphological features and general characteristics and laboratory data of diabetic patients

	Diabetes duration		Creatinine		Urine albumin-creatinine ratio	
	r	P	r	p	r	P
Endothelial Cell Density (cells/m^2)	−0.111	0.028	−0.063	0.21	−0.122	0.041
Average cell size (μm^2)	0.141	0.005	0.088	0.083	0.111	0.029
Coefficient of Variation(%)	0.094	0.044	0.042	0.461	0.121	0.017
Hexagonal cell ratio (%)	−0.058	0.093	−0.012	0.552	−0.034	0.198

DISCUSSION

In this study, we identified significantly decreased ECD and percentage of hexagonal cells, elevated average cell size and coefficient of variation in diabetic patients compared with the same parameters in healthy controls. Moreover, with an increase in the stage of DR, ECD and the percentage of hexagonal cells decreased while central corneal thickness increased. In this study, we determined a significant negative correlation between ECD and diabetes durationand the urinary albumin-creatinine ratio of patients in the diabetic group. In this study, unlike other studies, retinopathy nephropathy and keratopathy were evaluated together. The relationship between the severity of retinopathy and the cornea was evaluated, and a significant relationship was found.

The associations of corneal complications and instability of corneal endothelium in diabetic patients with excessive sorbitol accumulation in the corneal endothelium and accumulation of advanced glycation end products in the epithelial basement membrane have been known for years.[9,25] However, the data in the literature evaluating corneal morphological parameters in diabetic patients are conflicting, and these parameters may be associated with ethnic differences.

In this study, we determined a significant decrease in ECD values in diabetic patients compared with the values in age-matched controls, and there was a negative correlation between ECD and diabetes duration. The cornea is incapable of mitosis, and corneal endothelial cells have no regenerative capacity. For that reason, we can suggest that with an elongation in diabetic duration, the decrease in ECD cannot be restored. The only compensation mechanism at that point is the increased cellular pleomorphism and a decrease in the percentage of hexagonal cells. We also identified a correlation between average cell size and CV with diabetes duration, Similar to our results, Lee et al.[25] also reported decreased ECD and hexagonality in diabetic eyes with thicker corneas compared with the same parameters in healthy controls. Moreover, they also determined an augmentation in corneal morphological abnormalities in patients with a diabetic duration of over 10 years.

El-Agamy et al.[26] reported that ECD was significantly lower while CV was significantly higher in diabetic patients, but the differences in hexagonal cell percentage and CCT were not significantly different between diabetic patients and healthy controls. In a population-based cross-sectional study, Sudhir et al.[12] reported that ECD was significantly lower in diabetic patients than in controls, but there was no significant difference between groups regarding hexagonality % or CV of cell size.

Islam QU et al.[27] reported that the mean ECD was significantly lower in diabetic patients than in age-matched healthy controls, but the mean average cell size, CV and hexagonality were not significantly different between the two groups. Similar to our results, the duration of diabetes was significantly correlated with ECD and hexagonality. Choo et al.[28] reported that ECD and hexagonality significantly decreased, while average cell size and CV increased significantly in diabetic patients. However, they determined that there was no significant alteration in the CCT of patients with diabetes and that there was no correlation between corneal endothelial findings and the duration of diabetes or severity of diabetic retinopathy.

Leem et al.[29] reported that central corneal thickness was increased and ECD was decreased in patients with diabetes mellitus, and contact lens usage also significantly affected corneal morphology in diabetic patients. In our study, we did not include patients using contact lenses. In a prospective study, Storr-Paulsen et al.[30] reported that CCT was significantly higher in diabetic patients, but there were no significant differences between type 2 diabetic patients with good glycemic control and nondiabetic control subjects in ECD, hexagonality or CV values.

Microalbuminuria is an indicator of early cardiovascular death and progressive renal disease in diabetic patients.[31,32] In this study, we determined a significant correlation between the urine albumin-creatinine ratio and ECD, average cell size and CV. To the best of our knowledge, very few studies in the literature evaluating the association of microalbuminuria with ocular findings. In that respect, we can suggest that keratopathy should be evaluated in diabetic patients with more awareness.

Age is defined as the most important factor in evaluating corneal morphology, and it is known that with age, ECD decreases, and the corneal endothelium compensate by increasing the size. In this study, the diabetic and control groups were age matched. However, in subgroups of diabetes, age could not be matched, which may have affected the results and is one of the limitations of this study. There are also some other limitations of this study that should be mentioned. Although the determination of microalbuminuria with a single random urine sample is highly sensitive and specific, the gold standard in the diagnosis of microalbuminuria is an analysis of 24-h urine collection.

The corneal endothelium can be evaluated by specular microscopy. In the biomicroscopic examination of the eye, the fact that the cornea is transparent does not mean that the endothelium is normal. It may be useful for early diagnosis to direct patients with endothelial problems to nephrologists for clinical evaluation of nephropathy. In line with the data we obtained at the end of this study, diabetes mellitus reduces corneal endothelial functional reserve. Reduced endothelial reserve increases the risk of corneal endothelial damage in intraocular surgery.

Additionally, the risk of corneal decompensation should be kept in mind in diabetic patients as the severity of the disease increases. In diabetic patients with diabetic retinopathy and with nephropathy, caution should be exercised in terms of endothelial decompensation. To minimize endothelial damage during cataract surgery and other surgeries, if necessary, endothelial protective manoeuvres should be performed, and preoperative specular microscopy findings should be carefully examined in these patients.

CONCLUSIONS

In conclusion, keratopathy with decreased ECD and percentage of hexagonal cells and increased coefficient of variation is an important complication of type 2 diabetes. With an increase in the stage of DR, alterations in corneal findings also increased. There was a significant correlation between the urine albumin-creatinine ratio and ECD, average cell size and CV in diabetic patients. In that respect, we can suggest that keratopathy should be

evaluated more cautiously in diabetic patients. Larger, prospective studies are warranted to define the role of keratopathy in the long-term outcomes of diabetic patients

ABBREVIATIONS

DM: Diabetes Mellitus; DR: Diabetic retinopathy; ADA: American Diabetes Association; RBG: Random blood glucose; ECD: Endothelial cell density; CV: Coefficient of variation; CCT: Central corneal thickness

REFERENCES

1. Wild S., et al. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 27.5 (2004): 1047- 53.
2. Mohan V. et al. Epidemiology of type 2 diabetes: Indian scenario. Indian Journal of Medical Research 125.3 (2007): 217-30.
3. Molitch ME, Rutledge B, Steffes M, Cleary P. Renal insufficiency in the absence of albuminuria among adults with Type 1 diabetes in the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study; ADA Annual Meeting 2006 (Abstract 23-OR).
4. Kempen JH, O'Colmain BJ, Leske MC, Haffner SM, Klein R, Moss SE, Taylor HR, Hamman RF. Eye diseases prevalence research group. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol. 2004;122(4):552–63.
5. Hartnett ME, Baehr W, Le YZ. Diabetic retinopathy, an overview. Vis Res. 2017 Oct;139:1–6.
6. Shih KC, Lam KS, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. Nutr Diabetes. 2017;7(3):e251.
7. Bikbova G., et al. Corneal changes in diabetes mellitus. Current Diabetes Reviews 8.4 (2012): 294-302.
8. McCarey BE., et al. Review of Corneal Endothelial Specular Microscopy for FDA Clinical Trials of Refractive Procedures, Surgical Devices, and New Intraocular Drugs and Solutions. Cornea 27.1 (2008): 1-16.
9. Roszkowska AM., et al. Corneal endothelium evaluation in type I and type II diabetes mellitus. Ophthalmologica 213.4 (1999): 258- 61.
10. Inoue K., et al. The corneal endothelium and thickness in type II diabetes mellitus. Japanese Journal of Ophthalmology 46.1 (2002): 65-9.
11. Shenoy R., et al. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. European Journal of Ophthalmology 19.3 (2009): 369-75.
12. Sudhir RR., et al. Changes in the corneal endothelial cell density and morphology in patients with type 2 diabetes mellitus: a population-based study, SankaraNethralaya Diabetic Retinopathy and Molecular Genetics Study (SN-DREAMS, Report 23). Cornea 31.10 (2012): 1119-22.
13. Benetz B., et al. Specular microscopy. In: Krachmer J, Mannis M, Holland E. Cornea, 3rd ed. Philadelphia: Elsevier Mosby (2011): 178-98.
14. Tanimura K. A Quantitative Analysis of Corneal Endothelial Cells. Japanese Journal of Ophthalmology 32 (1981): 1835-39.
15. Weston BC., et al. Corneal hydration control in diabetes mellitus. Investigative Ophthalmology and Visual Science 36.3 (1995): 586- 95.
16. Su DW., et al. Diabetes, hyperglycemia, and central corneal thickness: the Singapore Malay Eye Study. Ophthalmology 115.6 (2008): 964-68.
17. Busted N., et al. Clinical observations on the corneal thickness and the corneal endothelium in diabetes mellitus. British Journal of Ophthalmology 65.10 (1981): 687-90.

18. Rao SK., et al. Corneal endothelial cell density and morphology in normal Indian eyes. Cornea 19.6 (2000): 820-23.
19. Yee R., et al. Changes in the Normal Corneal Endothelial Cellular pattern as a Function of Age. Current Eye Research 4.6 (1985): 671-8.
20. Maurice DM. Cellular membrane activity in the corneal endothelium of the intact eye. Experientia 24.11 (1968): 1094-95.
21. Ohno K., et al. Comparison of Recording Systems and Analysis Methods in Specular Microscopy. Cornea 18.4 (1999): 416-23.
22. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, et al. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology. 2003;110(9):1677 –82.
23. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(Suppl 1):S62 –9.
24. Lutty GA. Effects of diabetes on the eye. Invest Ophthalmol Vis Sci. 2013; 54(14):ORSF81 –7.
25. Lee JS, Oum BS, Choi HY, Lee JE, Cho BM. Differences in corneal thickness and corneal endothelium related to duration in diabetes. Eye (Lond). 2006 Mar;20(3):315 –8.
26. El-Agamy A, Alsubaie S. Corneal endothelium and central corneal thickness changes in type 2 diabetes mellitus. Clin Ophthalmol. 2017;11:481 –6.
27. Islam QU, Mehboob MA, Amin ZA. Comparison of corneal morphological characteristics between diabetic and nondiabetic population. Pak J Med Sci. 2017;33(6):1307 –11.
28. Choo M, Prakash K, Samsudin A, Soong T, Ramli N, Kadir A. Corneal changes in type II diabetes mellitus in Malaysia. Int J Ophthalmol. 2010;3(3):234 –6.
29. Leem HS, Lee KJ, Shin KC. Central corneal thickness and corneal endothelial cell changes caused by contact lens use in diabetic patients. Yonsei Med J. 2011 Mar;52(2):322 –5.
30. Storr-Paulsen A, Singh A, Jeppesen H, Norregaard JC, Thulesen J. Corneal endothelial morphology and central thickness in patients with type II diabetes mellitus. Acta Ophthalmol. 2014 Mar;92(2):158 –60.
31. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, Jensen G, Clausen P, Scharling H, Appleyard M, Jensen JS. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation. 2004;110(1):32 –5.
32. Xia F, Liu G, Shi Y, Zhang Y Impact of microalbuminuria on incident coronary heart disease, cardiovascular and all-cause mortality: a metaanalysis of prospective studies. Int J Clin Exp Med. 2015;8(1):1 –9.