Research Article

Sea Fish Consumption is Associated with Lower Risk of Diabetes Mellitus: A Cross-Sectional Study

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Abstract

The present study aimed to highlight the fasting and postprandial blood glucose levels among Sea Fish-Eaters (SFE) and Freshwater Fish-Eaters (FWFE) to establish the percentage of the existence of T2DM. The study was carried out among the sea fish-eaters both male and female (n=124) belonging to the community of Ramnagar block, Purba Medinipur district, and fresh water fish eaters (n=124) belonging to the community of Kotulpur block, Bankura district West Bengal, India. We collected data by directly interacting with the locals regarding the type of fish consumption for one week and correlated this with their fasting and postprandial blood glucose level measured using a glucometer. Among the SFE and FWFE participants the mean age of male and female is respectively 48.27±1.26 (n=30, 24.4%), 47.68±1.09 (n=40, 32.5%) and 56.09±1.23 (n=94, 94%), 52.33±1.06 (n=84, 67.5%). The mean fasting blood glucose level among female FWFE (121.41mg/dl) was significantly higher (P < 0.05) than female SFE (91.36mg/dl). The mean postprandial blood glucose level among female FWFE (196.13mg/dl) were also significantly higher (P < 0.05) than female SFE (140.05mg/dl). This study found that people who consume freshwater fish have a higher risk of T2DM than sea fish eaters. However, it remains unclear whether consumption of sea fish itself has a protective effect on T2DM or not we were not able to take a protective lifestyle into account in this study. To gather these findings we have to examine many other aspects.

Keywords: Diabetes Mellitus; Sea fish; Fresh water fish; Blood sugar

Abbreviations

IDF: International Diabetes Federation; T2DM: Type 2 Diabetes Mellitus; WHO: World Health Organization; PUFA: Polyunsaturated Fatty Acid; DHA: Docosahexaenoic acid; DPA: Docosapentaenoic Acid; EPA: Eicosapentaenoic Acid; STEPS: STEP Wise Approach to Surveillance; WC: Waist Circumference; HC: Hip Circumference; FBG: Fasting Blood Glucose; PPBG: Postprandial Blood Glucose; SE: Standard Error; SFE: Sea Fish-Eaters; FWFE: Freshwater Fish-Eaters

Introduction

According to the International Diabetes Federation (IDF), globally the number of people with Type 2 Diabetes Mellitus (T2DM) has risen rapidly from 425 million in 2017 to 463 million in 2019. It has also been estimated that the global burden of T2DM will further increase to more than 700 million by 2045. Globally, the prevalence of diabetes are found in the population is 8.9%. In India, the number of people with T2DM has drastically increased from 26 million in 1990 to 65 million in 2016. World Health Organization (WHO) ranked diabetes as the seventh leading cause of death in 2016 and estimated that 1 in 11 adults (20-79 years) has diabetes, 1 in 13 adults (20-79 years) has impaired glucose intolerance. Further WHO estimated that 2 out 3 people with diabetes live in urban areas [1-3]. T2DM is the most disastrous chronic metabolic disease characterized by a persistent state of hyperglycemia, inducing metabolic alteration, cell death, vascular complications, nephropathy, retinopathy, foot ulcer, endothelial dysfunction, dyslipidemia, an increase of oxidative stress, and severe inflammation resulting in high morbidity and mortality rates [4-7]. According to the estimation made by several studies in India that, for a low-income family with an adult suffering from diabetes, as much as 25% of family income could be devoted to diabetes care. The costs of diabetes affect everyone, everywhere, but is not the only crisis or financial problem. It also causes pain, anxiety, inconvenience, and generally lower quality of life [8]. International dietary recommendations suggest that regular fish consumption provides high-quality protein as well as essential nutrients useful for health and these benefits are the main drives for the population to buy fish [9-11]. Fish adds great nutritional value to the diet due to its content of long-chain omega-3 Polyunsaturated Fatty Acids (PUFA), such as Docosahexaenoic Acid (DHA), Docosapentaenoic Acid (DPA), and Eicosapentaenoic Acid (EPA), which are highly valued for their prophylactic and therapeutic properties in nutritional and health fields [12]. While fish, particularly oily fish, is generally considered to be an important part of a healthy diet and lowers the risk of diabetes [13,14]. This study aimed to identify associations between sea fish consumption and freshwater fish consumption concerning T2DM, with particular emphasis on detecting possible differences between sea fish eaters and freshwater fish eaters [15,16].

Materials and Methods

Study design and subjects

From November to December 2019, a population-based cross-sectional survey was carried out including the sea fish-eater community of Ramnagar (21°39'16.70"N, 87°29'19.94"E), Purba Medinipur district, West Bengal, India, and freshwater fish-eater

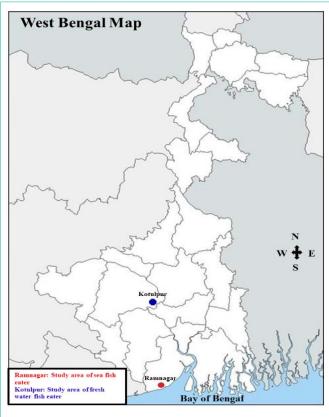


Figure 1: Study places of West Bengal.

community of Kotulpur (22°59'12.91"N, 87°32'34.81"E), Bankura, West Bengal, India (Figure 1). The study was conducted by the declaration of Helsinki, and all procedures were approved by the ethics committee of Raja Narendra Lal Khan Women's College (Autonomous). All participants (age ranging from 21 to 60 years) signed informed consent before data collection. Subjects with serious comorbid diseases like severe infection, stroke, myocardial infarction, major surgery, malabsorption, history of using drugs significantly affecting glucose metabolism (glucocorticoids, oral contraceptives containing levonorgestrel or high dose estrogen, phenytoin, and high dose thiazide diuretics, etc.) and pregnant women were excluded for the study. We collected data by directly interacting with locals regarding types of fish consumption for a week and collected fasting and postprandial blood glucose by glucometer. Sea fish eaters consume mainly Panna microdon (Bleeker, 1849), the local name 'volavetki'; Coilia dussumieri (Valenciennes, 1848) local name 'ruli'; Opisthopterus tardoore (Cuvier, 1829) local name 'tapra'; Trichiurus lepturus (Linnaeus, 1758) local name 'rupapatia'; Setipinna phasa (Hamilton, 1822) local name 'phansa' and fresh water fish eating section mainly consume and Labeo bata (Hamilton,1822), local name 'bata'; Opisthopterus tardoore (Cuvier,1829), local name 'folui'; Amblypharyngodon mola (Hamilton, 1822), local name 'mourala'; Labeo rohita (Hamilton, 1822) local name 'ruhu'; Catla catla (Hamilton, 1822) local name 'katla' [17,18]. The sample for participants was obtained by using a formula with the following parameters: 8.9% prevalence of diabetes (P), 5% margin of error (E), and a standard normal deviation (Z) of 1.96 [19].

Formula:
$$N = \frac{z^2 p(1-P)}{E^2}$$

Measurement of waist-hip ratio

The WHO STEP wise approach to surveillance (STEPS) recommended a stretch-resistant tape for measuring Waist Circumference (WC) and Hip Circumference (HC). WC (cm) was measured at the approximate mid-point between the lower margin of the last palpable rib and the top of the iliac crest. HC (cm) was measured by the passing tape at the point of the tochanteron (head of the femur).

Waist-hip ratio = WC (cm) / HC (cm)

Waist-hip ratio for men > 0.9 and women > 0.85 was considered risk of metabolic syndrome like T2DM [20].

Biochemical analysis of fasting and postprandial blood glucose level

During the survey, we requested the subjects to collect blood for checking and Fasting Blood Glucose (FBG) and Postprandial Blood Glucose (PPBG) levels. Therefore, we collected the blood by pricking the finger, and a drop of blood was added the glucometer strip and the fasting blood glucose level was recorded. Diabetes was considered above 126mg/dl of FBG. To measure PPBG subjects were directed to inform the lunchtime, we collected blood after 2 hours of lunch, and above 200mg/dl of PPBG was considered diabetes [21,22].

Statistical analysis

Data were demonstrated as mean \pm standard deviation (SD). Mean values were tested by a one-way analysis of variance model using the origin package for windows version 6.1 [23].

Results

The study was conducted on the adult population (age group 21 to 60 years) including both male and female individuals from where 124 sea fish-eaters (SFE) in Ramnagar (Purba Medinipur district), West Bengal, and another 124 Freshwater Fish-Eaters (FWFE) in katulpur, Bankura, West Bengal were selected for the study. Among the SFE participants, the mean age of male is 48.27±1.26 (n=30, 24.4%) and female is 56.09±1.23 (n=94, 94%). Another data found that the FWFE participants, the mean age of male is 47.68±1.09 (n=40, 32.5%) and female is 52.33±1.06 (n=84, 67.5%; Figure 2). Mean value of age was not significant different (P>0.05). In terms of educational qualifications, 6.7% male SFE, 8.5 % female SFE, 4% male FWFE, and 7.1 % female FWFE are illiterate. It was also found that 73.3 % male SFE, 76.7% female SFE, 6.5% male FWFE, and 78.6% female FWFE received primary education. Further information indicates that 13.3% male SFE, 10.6% female SFE, 15% male FWFE, and 9.6% female FWFE having secondary and higher secondary qualifications. Some more information was found that 6.7% male SFE, 4.2% female SFE, 7.5% male FWFE, and 4.7% female FWFE are qualified for college and university level degrees. Among the female participants, most of them were housewives 85.1% SFE and 83.3% FWFE. The majority of male participants are daily laborers or possess small businesses. Among the SFE participants, 83.4% male and 89.3% female consume sea fish daily, 10% male and 5.4% female consume sea fish more than or equal to two days per week. Another data found that among the FWFE participants, only 10% male and

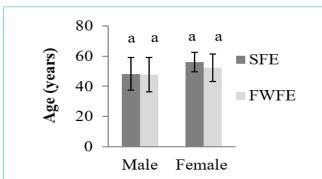


Figure 2: Mean age of sea fish eater (SFE) and freshwater fish eater (FWFE) in groups of male and female. Data are mean ± standard deviation. a, a mean value was not significant different (P>0.05).

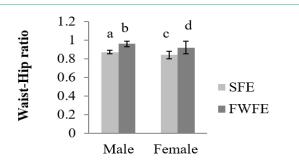


Figure 3: Mean Waist-Hip ratio of sea fish eater (SFE) and freshwater fish eater (FWFE) in groups of male and female. Data are mean \pm standard deviation. a, b and, c, d mean value was significant different (P <0.05).

6% female consume freshwater fish daily and 80% male and 86.9% female consume freshwater fish more than or equal to two days per week (Table 1). Mean value of waist-hip ratio of FWFE (both male and female) was significantly higher than SFE (both male and female) separately (male vs male and female vs female; Figure 3). The mean fasting blood glucose level among female FWFE is 121.41 mg/dl were significantly higher (P <0.05) than female SFE 91.36mg/dl. There is no significant difference (P >0.05) among male SFE 84.2mg/dl, female SFE 91.36mg/dl and male FWFE 90.92mg/dl (Figure 4a). The mean postprandial blood glucose level among female FWFE (196.13mg/dl) was significantly higher (P < 0.05) than female SFE (140.05mg/dl). There is no significant difference (P >0.05) among male SFE 124.6mg/dl, female SFE 140.05mg/dl and male FWFE 139.25mg/dl (Figure 4b). Figure 5 showed that FWFE female and male participants are higher percentage of T2DM than SFE female and male participants.

Discussion

The present study was mainly a population-based cross-sectional study to correlate the blood glucose level among SFE and FWFE and the existence (percentage among participants) of T2DM [24]. We found a normal blood glucose level among SFE in both male and female participants. It remains unclear whether sea fish is itself has a protective effect on T2DM or we were not able to take a protective lifestyle or any other dietary information should be incorporated into account in the present study [15,16,25]. SFEs were characterized by a high intake of sea fish such as volavetki, ruli, tapra, Rupapatia, and Phansa. They mainly reside near the coastal area of the Bay of Bengal in Ramnagar (Purba Medinipur district). The following quotes provide examples of participants' perspectives on sea fish consumption: "We

Table 1: Sociodemographic characteristics of the participants.

Sociodemographic profile	Sea fish eater				Fresh water fish eater			
Gender	Male ¹	(%)2	Female ¹	(%)2	Male ¹	(%)2	Female ¹	(%)2
No. of participant	30	24.4	94	75.6	40	32.5	84	67.5
Age (years)								
21-40	8	26.7	24	25.9	11	27.5	22	26.2
41-60	22	73.3	70	74.1	29	72.5	62	73.8
Level of education								
Never went to school	2	6.7	8	8.5	4	10	6	7.1
Primary school	22	73.3	72	76.7	27	67.5	66	78.6
Secondary school	4	13.3	10	10.6	6	15	8	9.6
College/ university	2	6.7	4	4.2	3	7.5	4	4.7
Employment status								
House wife			80	85.1			70	83.3
Employed	27	90	10	10.5	36	90	8	9.5
Unemployed	3	10	4	4.4	4	10	6	7.2
Fish consumption								
Daily	25	83.4	84	89.3	4	10	5	6
≥2 days per week	3	10	5	5.4	32	80	73	86.9
Weekly	1	3.3	3	3.1	3	7.5	4	4.7
No consumption	1	3.3	2	2.2	1	2.5	2	2.4

¹Number; ²Percentage (%).

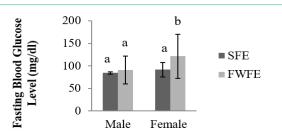


Figure 4a: Mean Fasting Blood Glucose Level (mg/dl) of sea fish eater (SFE) and freshwater fish eater (FWFE) in groups of male and female. Data are mean \pm standard deviation. a) A mean value was not significant different (P >0.05) and a and b mean value was significantly different (P <0.001).

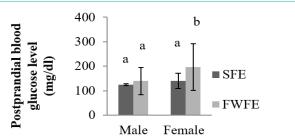


Figure 4b: Mean postprandial blood glucose level (mg/dl) of sea fish eater (SFE) and freshwater fish eater (FWFE) in groups of male and female. Data are mean ± standard error. a) A mean value was not significant different (P >0.05) and a and b mean value was significantly different (P <0.001).

are buying sea fishes because it's abundantly available in the local market at the lowest rate and we are not aware of any information regarding nutritional benefits". High postprandial blood glucose levels and waist-hip ratio are existing among FWFE females representing

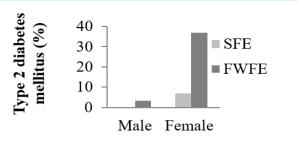


Figure 5: The percentage of Type 2 diabetes mellitus of sea fish eater (SFE) and freshwater fish eater (FWFE) in groups of male and female.

T2DM (37%). They never eat sea fish. They are usually buying freshwater fish due to its availability and nonavailability of sea fish. FWFEs were characterized by a high intake of freshwater fish such as bata, folui, mourala, Ruhu, and Katla [18]. They mainly reside in katulpur region, Bankura. The following quotes provide an example of participants' perspectives on freshwater fish consumption: "We are buying freshwater fish become it's available in our local market". The long-chain n-3 PUFA particularly DHA and EPA is abundant in sea fish [26,27]. So, sea fish consumption is reversibly associated with glycemia [28-30]. A positive effect of n-3 PUFA on insulin sensitivity has also been established by an animal experimental study [31-33]. Sea fish oil was associated with increase fasting insulin in the human model representing its benefits among diabetic patients [34]. Salmon (Sea fish of Alaska) oil consumption was associated with a lower risk of impaired glucose tolerance [35,36]. The study investigated the cross-sectional association between the habitual consumption of sea fishes or freshwater fishes and blood glucose levels in a population of men and women without self-reported diabetes [37]. There was evidence that women who have never consumed sea fish had higher fasting and postprandial blood glucose than women who consume sea fish [38,39].

Conclusion

The relationship between sea fish consumption and lower risk of T2DM is generally well accepted, but the mechanism for this effect is not fully understood. The potential hypoglycemic benefits of sea fish may be due to the presence of nutrients mainly n-3 PUFA and others components that will be required for further research. So, future research is warranted to better elucidate the mechanism of action and composition of sea fishes for potential health benefits for the antidiabetic activity of sea fishes.

References

- 1. International Diabetes Federation, Diabetes Facts and Figures. 2021.
- 2. Karuranga S, Malanda B, Saeedi P, Salpea P. IDF DIABETES ATLAS, Ninth edition. Online version of IDF Diabetes Atlas. 2019.
- Tandon N, Anjana RM, Mohan V, Kaur T, Afshin A, Ong K, et al. The increasing burden of diabetes and variations among the states of India: the Global Burden of Disease Study 1990-2016. The Lancet Global Health. 2016; 6: e1352-e1362.
- Zheng J, Cheng J, Zheng S, Feng Q, Xiao X. Curcumin, a polyphenolic curcuminoid with its protective effects and molecular mechanisms in diabetes and diabetic cardiomyopathy. Front Pharmacol. 2018; 9: 472.
- Lu M, Yin N, Liu W, Cui X, Chen S, Wang E. Curcumin Ameliorates Diabetic Nephropathy by Suppressing NLRP3 Inflammasome Signaling. Biomed Res Int. 2017; 2017: 1516985.

- Maugeri A, Mazzone MG, Giuliano F, Vinciguerra M, Basile G, Barchitta M, et al. Curcumin Modulates DNA Methyltransferase Functions in a Cellular Model of Diabetic Retinopathy. Oxid Med Cell Longev. 2018; 2018: 5407482.
- Roxo DF, Arcaro CA, Gutierres VO, Costa MC, Oliveira JO, Lima TFO, et al. Curcumin combined with metformin decreases glycemia and dyslipidemia, and increases paraoxonase activity in diabetic rats. Diabetol Metab Syndr. 2019; 11: 33.
- World Health Organization. Global report on diabetes. World Health Organization, 2016.
- Torrissen JK, Onozaka Y. Comparing fish to meat: perceived qualities by food lifestyle segments. Aquaculture Economics & Management. 2017; 21: 44-70.
- Kim YS, Xun P, He K. Fish consumption, long-chain omega-3 polyunsaturated fatty acid intake and risk of metabolic syndrome: a meta-analysis. Nutrients. 2015; 7: 2085-2100.
- Torris C, Molin M, Cvancarova Smastuen M. Fish consumption and its possible preventive role on the development and prevalence of metabolic syndrome - a systematic review. Diabetology & Metabolic Syndrome. 2014; 6: 112.
- Bonilla-Mendez JR, Hoyos-Concha JL. Methods of extraction, refining and concentration of fish oil as a source of omega-3 fatty acids. Corpoica Cienciay Tecnologia Agropecuaria. 2018; 19: 645-668.
- Patel PS, Sharp SJ, Luben RN, Khaw KT, Bingham SA. Association between type of dietary fish and seafood intake and the risk of incident type 2 diabetes: the European prospective investigation of cancer (EPIC)-Norfolk cohort study. Diabetes Care. 2009: 32: 1857-1863.
- Panagiotakos DB, Zeimbekis A, Boutziouka V, Economou M, Kourlaba G. Long-term fish intake is associated with better lipid profile, arterial blood pressure, and blood glucose levels in elderly people from Mediterranean islands (MEDIS epidemiologivan cal study). Med Sci Monit. 2007; 13: CR307-CR12.
- Tripura K, Kaushik Nag K, Karmakar N, Datta A, Bhattacharjee P. A cross sectional study on the prevalence of diabetes mellitus among adult population in a peri-urban area of West Tripura, India. International Journal of Research in Medical Sciences. 2019; 7: 843-848.
- Kumar NK, Katkuri S, Ramyacharitha I. A study to assess prevalence of diabetes mellitus and its associated risk factors among adult residents of rural Khammam. International Journal of Community Medicine and Public Health. 2018; 5: 1360-1365.
- Talwar PK, Jhingran AG. Inland Fishes of India and Adjacent Countries. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi, India. 1991; 1: 541.
- Ghorai SK, Bera Sk, Jana D, Mishra S. Status of the largest dry fish market of East India: A study on Egra Regulated Dry Fish Market, Egra, Purba Medinipur, West Bengal. International Journal of Current Research and Academic Review. 2014; 2: 54-65.
- Kasole R, Martin HD, Kimiywe J. Traditional Medicine and Its Role in the Management of Diabetes Mellitus: "Patients' and Herbalists' Perspectives". Evidence-Based Complementary and Alternative Medicine. 2019; 2835691.
- World Health Organization. Waist Circumference and Waist-Hip Ratio: Report of a WHO Expert Consultation. World Health Organization. 2008.
- 21. Pickering D, Marsden J. How to measure blood glucose. Community Eye Health. 2014; 27: 56-57.
- World Health Organization. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia Report of a WHO/IDF Consultation. International Diabetes Federation. 2006.
- 23. Zhang FL, Ren JX, Zhang P, Jin H, Qu Y, Yu Y, et al. Strong Association of Waist Circumference (WC), Body Mass Index (BMI), Waist-to-Height Ratio (WHtR), and Waist-to-Hip Ratio (WHR) with Diabetes: A Population-Based Cross-Sectional Study in Jilin Province, China. Journal of Diabetes Research. 2021; 2021: 8812431.
- 24. Rylander C, Sandanger TM, Engeset D, Lund E. Consumption of Lean Fish Reduces the Risk of Type 2 Diabetes Mellitus: A Prospective Population

Based Cohort Study of Norwegian Women. PLoS ONE. 2014; 9: e89845.

- 25. Yin X, Chen Y, Lu W, Jin T, Lin L. Association of dietary patterns with the newly diagnosed diabetes mellitus and central obesity: a community based cross-sectional study. Nutrition and Diabetes. 2020; 10: 16.
- Alqathama A, Alluhiabi G, Baghdadi H, Aljahani L, Khan O, Jabal S, et al. Herbal medicine from the perspective of type II diabetic patients and physicians: what is the relationship? BMC Complement Med Ther. 2020; 20: 65
- Torris C, Molin M, Cvancarova SM. Lean fish consumption is associated with lower risk of metabolic syndrome: a Norwegian cross sectional study. BCM Public Health. 2016: 16: 347.
- 28. Harding AH, Day NE, Khaw KT, Bingham SA, Luben RN, Welsh A, et al. Habitual fish consumption and glycated haemoglobin: The EPIC-Norfolk Study. Eur J Clin Nutr. 2004; 58: 277-284.
- McDonnell SL, French CB, Baggerly CA, Harris WS. Cross-sectional study of the combined associations of dietary and supplemental eicosapentaenoic acid + docosahexaenoic acid on Omega-3 Index. Nutr Res. 2019; 71: 43-55.
- Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ. Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. Am J Clin Nutr. 2002; 76: 1007-1015.
- 31. Lalia AZ, Lanza IR. Insulin-Sensitizing Effects of Omega-3 Fatty Acids: Lost in Translation? Nutrients. 2016; 8: 329.
- 32. El Bilbeisi AH, Hosseini S, Djafarian K. Association of dietary patterns with diabetes complications among type 2 diabetes patients in Gaza Strip, Palestine: a cross sectional study. J Health Popul Nutr. 2017; 36: 37.

- Chewcharat A, Chewcharat P, Rutirapong A, Papatheodorou S. The effects of omega-3 fatty acids on diabetic nephropathy: A meta-analysis of randomized controlled trials. PLoS One. 2020; 15: e0228315.
- 34. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chisholm DJ, Campbell LV. The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. N Engl J Med. 1993; 328: 238-244.
- 35. Filipovic MG, Aeschbacher S, Reiner MF, Stivala S, Gobbato S, Bonetti N, et al. Whole blood omega-3 fatty acid concentrations are inversely associated with blood pressure in young, healthy adults. Journal of hypertension. 2018; 36: 1548-1554.
- 36. Anjana RM, Deepa M, Pradeepa R, Mahanta J, Narain K, Das HK, et al. ICMR-INDIAB Collaborative Study Group. Prevalence of diabetes and prediabetes in 15 states of India: results from the ICMR-INDIAB populationbased cross-sectional study. Lancet Diabetes Endocrinol. 2017; 5: 585-596.
- 37. Cassidy S, Chau JY, Catt M, Bauman A, Trenell M. Cross-sectional study of diet, physical activity, television viewing and sleep duration in 233 110 adults from the UK Biobank; the behavioural phenotype of cardiovascular disease and type 2 diabetes BMJ Open. 2016; 6: e010038.
- Osonoi Y, Mita T, Osonoi T, Saito M, Tamasawa A, Nakayama S, et al. Relationship between dietary patterns and risk factors for cardiovascular disease in patients with type 2 diabetes mellitus: a cross-sectional study. Nutr J. 2015; 15: 15.
- Khalaf AJ, Whitford DL. The use of complementary and alternative medicine by patients with diabetes mellitus in Bahrain: a cross-sectional study. BMC Complement Altern Med. 2010; 10: 35.