

# A Comparison of Gallbladder Contraction Index in Diabetics and Non diabetics using Ultrasonogram: A Cross-sectional Study

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## ABSTRACT

**Introduction:** In India, the prevalence of gallstones is considered as a major public health problem and among diabetic patients the prevalence is still more increasing. Presence of Diabetic Autonomic Neuropathy (DAN) is a triggering factor for gallbladder diseases among diabetic patients.

**Aim:** To compare the gallbladder volume and contraction index between diabetic patients and non diabetic healthy controls.

**Materials and Methods:** A cross-sectional study was conducted in a tertiary care hospital at Chennai for a period of six months from June 2019 to December 2019. The total 90 subjects were divided into two groups of 45 each, group A consist of 45 known Type I or Type II diabetes mellitus (DM) and group B with 45 non diabetic healthy controls. The gallbladder volume was assessed initially in the fasting state, then the patient was asked to have fatty meal and again the gallbladder volume was measured after two hours for all the patients and the gallbladder contraction index was calculated using

these values. Autonomic neuropathy grading was done after performing neuropathy assessment tests and the association between the gallbladder contraction index and grading of autonomic neuropathy was analysed.

**Results:** The mean fasting and postprandial gallbladder volume were much higher among diabetic patients compared to non diabetic controls and similarly the gallbladder contraction index was lesser among diabetic patients compared to the controls ( $16.6 \pm 5.9 \text{ cm}^3$ ,  $4.03 \pm 1.8 \text{ cm}^3$ ) and ( $32.08 \pm 11.7 \text{ cm}^3$ ,  $17.4 \pm 10.6 \text{ cm}^3$ ) the difference was found to be statistically significant ( $p\text{-value} < 0.001$ ). The grading of autonomic neuropathy was inversely proportional to gallbladder contraction index and it was found to be statistically significant ( $p\text{-value} < 0.001$ ).

**Conclusion:** The gallbladder volume was high and the contraction index was less among the diabetic patients with neuropathy compared to non diabetic patients and so a regular screening for gallbladder using ultrasonogram is recommended for long standing diabetic patients with neuropathy.

**Keywords:** Diabetic autonomic neuropathy, Diabetes mellitus, Gallbladder volume, Gallstones

## INTRODUCTION

Diabetes mellitus is a type of metabolic disorder characterised by increase in blood glucose level due to defect in the secretion of insulin, action of insulin or combination of both. The prevalence of diabetes is increasing year by year both in developed and developing countries. The recent study shows the prevalence of diabetes globally is 9.3% per 2019 records and the estimate for 2030 is 10.2% [1-3]. It is predicted that by 2030 diabetes mellitus may afflict up to 80 million individuals in India [4-6].

Among various complications related to diabetes, diabetic neuropathy is the most common and serious complication. Diabetic Autonomic Neuropathy (DAN) is among the least recognised and understood complications of diabetes despite having a significant negative impact on survival and quality of life in people with diabetes [7,8]. DAN most of the time presents as a subclinical manifestation rather than a clinical evidence. As because vagus nerve accounting for more than 75% of the parasympathetic activity and this becomes the most common nerve to be involved first in autonomic neuropathy. Though the exact pathogenesis of DAN is not clear, metabolic insult to nerve fibers due to advanced glycosylation end products, neurovascular insufficiency, autoimmune damage and neurohormonal growth factor deficiency were some of the postulated hypothesis [9]. The common clinical signs and symptoms related to gastrointestinal system due to DAN are oesophageal dysmotility, gastroparesis diabetorum, constipation, diarrhoea, fecal incontinence and gallbladder atony and enlargement [10].

In India, prevalence of gallstones is considered as a major public health problem. The prevalence varied in different region and it was reported that the prevalence was higher in North Indian population

compared to South Indian. It also highlighted that, the prevalence was increasing with age with a maximum occurrence in the 6th decade [11,12]. Generally, gallbladder diseases are more frequent in females compared to males due to hormonal factors. Increasing age, overweight, family history of gall stone disease and type 2 DM were found to be the risk factors for gallbladder diseases [13]. Several studies done across the world had showed an increased prevalence of gallbladder stones in patients with DM ranging from 20-32% [14]. A recent study done in Italy compared the prevalence of gallstone between diabetic patients and general population and found the prevalence to be significantly higher in diabetic patients (24.8% vs 13.8%) [15]. The study further showed that the duration of diabetes had a positive correlation with the prevalence of gallstones and the fasting blood sugar levels showed an inverse relationship with the prevalence of gall stones. Though the pathogenesis of gallbladder dysfunction in diabetes is not fully understood, the only possible hypothesis was a decrease in Cholecystokinin (CCK) from small intestine due to gastroparesis caused by autonomic neuropathy, resulting in gallbladder stasis and eventually leading to cholesterol crystal precipitation and gall stone formation [16,17]. As of today not much studies have been conducted in India to assess the gallbladder functions among diabetic patients and so, the present study aimed to compare the gallbladder volume and contraction index between diabetic patients and non diabetic population as these two factors influence the gallstone formation.

## MATERIALS AND METHODS

A cross-sectional comparative study was conducted in a tertiary care hospital at Chennai for a period of six months from June 2019 to December 2019. The study was started after getting approval

from the Institutional Ethical Committee and the informed consent was obtained from all the study participants.

**Sample size calculation:** With a significance level  $\alpha$  of 0.05 and power  $(1-\beta)$  of 0.8, and with a sensitivity of 0.94, the minimum required sample size was calculated as 90 subjects using the formula given below

$$N_A = kn_B \text{ and } n_B = (1+1/K) (\Omega Z_{1-\alpha/2} + Z_{1-\beta} / \mu_A - \mu_B)^2$$

The total 90 subjects were divided into two groups of 45 each, group A consist of 45 known type I or type II diabetes mellitus and group B with 45 non diabetic healthy controls.

**Inclusion criteria:** The inclusion criteria for group A is patients with a history of diabetes for more than 3 years duration and for group B patients, healthy individuals aged more than 30 years without diabetes.

**Exclusion criteria:** Patients with known hepatobiliary diseases, like cholelithiasis, chronic cholecystitis, ascending cholangitis, cirrhosis of liver, obese patients with BMI more than 30, patients with history of dyslipidemia and cerebrovascular accidents were excluded from the study as these factors might interfere with gallbladder contraction.

All the study subjects were kept fasting for six hours before scanning. Ultrasound scanning was done for all the 90 study subjects using Voluson S6 GE ultrasound machine with 4MHz curvilinear probe. The gallbladder volume was assessed initially in the fasting state, then the patient was asked to have fatty meal and again the gallbladder volume was measured after two hours. The greatest length (Length), maximum transverse diameter (Width), and the maximum anteroposterior diameter (Height) were measured, three measurements were taken for all these parameters. The ultrasound images were interpreted by two Radiologists. The fasting gallbladder volume was assessed by one Radiologist and the post prandial volume by another Radiologist. Both of them were blinded to the medical records. The gallbladder volume was calculated using the formula.

$$\text{Volume} = (\text{length} \times \text{width} \times \text{height}) \times 0.52$$

The gallbladder (GB) contraction index was calculated by the formula [18]:

$$\frac{(\text{Fasting GB volume} - \text{Postprandial GB volume}) \times 100}{\text{Fasting GB volume}}$$

The gallbladder contraction index was compared with the grading for autonomic neuropathy. The following were the test performed to assess the grading of autonomic neuropathy:

1. Heart rate variation after immediate standing-less than 10 is considered abnormal.
2. Blood pressure response to standing after two minutes- sustained drop systolic BP >20 mmHg and sustained drop in diastolic BP more than 10 mmHg without an appropriate increase in heart rate is considered abnormal.
3. Blood pressure response to sustained hand grip for two minutes- a drop in diastolic BP more than 10 mmHg is considered abnormal.
4. Heart rate variation to deep breathing for six seconds-variation less than 10 is considered abnormal.
5. Pupillary response to light-dilated pupil with sluggish response is considered abnormal.

The grading was done as follows

Grade 0-No test abnormal

Grade 1-One test abnormal

Grade 2-Two test abnormal

Grade 3-Three or more test abnormal [19]

## STATISTICAL ANALYSIS

All the data were entered and analysed using SPSS version 24. Mean

and standard deviation were derived for all the parametric variables and the percentage was calculated for frequency variables. Chi-square test was used to derive the statistical inference between the two variables. A p-value <0.05 was found to be statistically significant.

## RESULTS

The age and gender wise distribution of the study subjects in the present study shows that majority of them were in the age group between 30 and 50 years and there was almost equal distribution of males and females in all age groups both among cases and controls [Table/Fig-1]. Among the cases, 24.4% were having type I diabetes mellitus and the remaining 75.6% were type II diabetic patients. The duration of diabetes for majority of the subjects was between 5 and 10 years [Table/Fig-2]. The mean fasting and postprandial blood sugar among cases and controls was shown in [Table/Fig-3] and statistical significant difference was observed between the two groups. The mean fasting gallbladder volume was significantly high among diabetic patients compared to non diabetics and the gallbladder contraction index was found to be significantly less among the cases compared to the controls [Table/Fig-4]. Among the diabetic patients, signs of autonomic neuropathy were tested and graded accordingly and it was found that the gallbladder volume was found to be increasing both in fasting and postprandial states as the grading of autonomic neuropathy increases. Similarly, the grading of autonomic neuropathy was inversely proportional to gallbladder contraction index and it was found to be statistically significant [Table/Fig-5]. The ultrasonogram pictures of measuring

Age group (Years)	Cases (n=45)		Controls (n=45)	
	Males n (%)	Females n (%)	Males n (%)	Females n (%)
20-30	6 (26.08%)	9 (40.9%)	4 (14.8%)	6 (33.33%)
31-40	5 (21.73%)	3 (13.63%)	6 (22.2%)	5 (27.77%)
41-50	6 (26.08%)	6 (27.27%)	9 (33.3%)	5 (27.77%)
51-60	3 (13.04%)	2 (9.09%)	6 (22.2%)	1 (5.55%)
61-70	2 (8.69%)	2 (9.09%)	1 (3.7%)	1 (5.55%)
>70	1 (4.34%)	0	1 (3.7%)	0
Total	23 (100%)	22 (100%)	27 (100%)	18 (100%)
Mean±SD	46.3±6.2		43.7±8.1	

[Table/Fig-1]: Age and gender wise distribution of the study subjects.

Duration of diabetes (Years)	Type of diabetes	
	Type I n (%)	Type II n (%)
<5	2 (18.18%)	9 (26.4%)
5-10	6 (54.54%)	21 (61.7%)
10.1-15	1 (9.09%)	4 (11.7%)
15.1-20	2 (18.18%)	0
Total	11 (100%)	34 (100%)

[Table/Fig-2]: Distribution of the cases based on the type of diabetes and duration of diabetes.

Blood sugar (mg/dL)	Cases (mean±SD)	Controls (mean±SD)	p-value
Fasting blood sugar	126±20.8	82±13.9	<0.001
Postprandial blood sugar	189±39.7	108±19.4	<0.001

[Table/Fig-3]: Mean fasting and postprandial blood sugar levels between cases and controls.

p-value derived by applying Student's T-test; p-value denotes significant

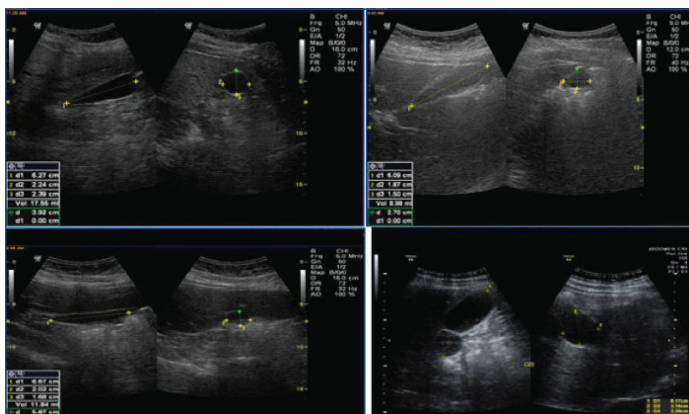
Gallbladder volume and index	Cases (mean±SD)	Controls (mean±SD)	p-value
Fasting gallbladder volume (cm³)	32.08±11.7	16.6±5.9	<0.001
Postprandial gallbladder volume (cm³)	17.4±10.6	4.03±1.8	<0.001
Gallbladder contraction index (%)	51.6±19	77.1±6.3	<0.001

[Table/Fig-4]: Gallbladder volume and contraction index between cases and controls. p-value derived by applying Student's T-test; p-value denotes significant

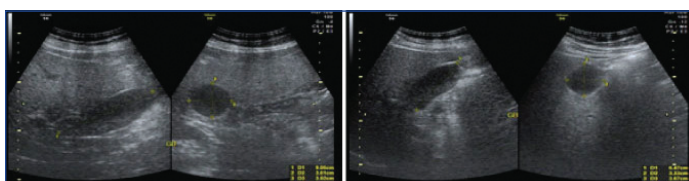
Grading of autonomic neuropathy	Fasting gallbladder volume (cm <sup>3</sup> ) (mean±SD)	Postprandial gallbladder volume (cm <sup>3</sup> ) (mean±SD)	Gallbladder contraction index (%) (mean±SD)
Grade 0 (n=14)	19.2±3.5	4.3±1.3	77.2±6.8
Grade 1 (n=17)	34.6±6.3	20.1±3.7	42.9±4.7
Grade 2 (n=10)	41.1±4.8	26.4±3.9	35.4±3.6
Grade 3 (n=4)	54.6±5.4	41.6±4.2	26.9±3.0
p-value	<0.001	<0.001	<0.001

**[Table/Fig-5]:** Gallbladder volume and contraction index according to grading of autonomic neuropathy among the cases.

p-value derived using ANOVA; p-value denotes significant



**[Table/Fig-6]:** Ultrasonogram images showing the measurements of gallbladder volume and gallbladder contraction index.



**[Table/Fig-7]:** Ultrasonogram images showing gallbladder volume measurements among patients with grade II and grade III autonomic neuropathy.

the gallbladder volume and contraction index for few patients was shown in [Table/Fig-6,7].

## DISCUSSION

The DAN of the gastrointestinal system takes several forms such as oesophagopathy, gastroparesis; enteropathy; and biliary tract disorders. One of the commonest biliary tract disorders due to DAN is gallstone formation [20]. Literatures had quoted the following reasons as the most common cause for gallstone formation in diabetic patients: decreased motility of gallbladder, reduced release of postprandial Cholecystokinin (CCK), reduced sensitivity of gallbladder smooth muscle to CCK along with decreased CCK receptors in the gallbladder wall, bile supersaturation, and sometimes the presence of gall stones themselves [21]. Gallbladder innervation is mediated by both sympathetic and parasympathetic nervous system. The parasympathetic system causes gallbladder contraction and the sympathetic system causes relaxation. During every meal the release of CCK mediates the parasympathetic system and causes the gallbladder contraction. In DAN patients, the motility defects occur in gallbladder which would manifest as increased fasting volume, decreased ejection fraction which would lead to decreased rate of ejection and increased residual volume of the gallbladder [22]. The present study aimed to compare the gallbladder volume and contraction index between the diabetic patients with autonomic neuropathy and non diabetic controls. The results of this study showed an increase of fasting gallbladder volume among diabetes group compared to non diabetic group and it was in par with the previous studies where they also showed a decrease in gallbladder emptying which is a proxy measure for gallbladder motility. These studies further inferred that autonomic neuropathy is

the reason for reduced gallbladder motility which causes decreased ejection fraction of gallbladder and leads to increased gallbladder volume. In the present study, due to logistic reasons we haven't

Author name	Diabetics with neuropathy Gallbladder volume in cm <sup>3</sup>	Non diabetic patients Gallbladder volume in cm <sup>3</sup>
Agarwal AK et al., [20]	53.88	28.16
Singh S et al., [21]	49.75	21.90
Raman PG et al., [22]	26.82	18.3
Present study	32.0	16.8

**[Table/Fig-8]:** Comparison of gallbladder volumes reported in previous studies with that of present study.

studied the gallbladder emptying time. Below is a table showing the comparison of the gallbladder volume between the two groups in previously done studies and the present study [Table/Fig-8].

Similarly in the present study, the postprandial gallbladder volume was also found to be increased among diabetic patients compared to non diabetic controls. No previous studies were found measuring the gallbladder volume in the postprandial period, all studies had only measured the volume in the fasting stage.

In healthy individuals, during postprandial period there is an increase in the CCK levels favouring the gallbladder emptying time, whereas in diabetic patients with autonomic neuropathy the sensitivity of gallbladder to CCK is impaired and so the emptying time is delayed and invariably leading to increase in the gallbladder volume [23-26]. As because both the fasting and postprandial gallbladder volume is high among the diabetic patients the gallbladder contraction index was found to be less in diabetic patients compared to non diabetic controls. Further in this study, we also proved that the grading of autonomic neuropathy is directly proportional to gallbladder volume and indirectly proportional to gallbladder contraction index and so, we can readily hypothesise that autonomic neuropathy is a major cause of diabetic cholecystopathy as mentioned in previous literature [27-29]. However, autonomic neuropathy cannot entirely account for the cholecystoparesis. A study done by Annese V et al., have shown that autonomic neuropathy has a poor predictive value for motor disorders, suggesting the co-existence of other pathophysiologic mechanisms such as delayed gastric emptying and decreased alpha-adrenergic tone in gallbladder along with increased bile lithogenicity [28].

Previous studies had shown a positive correlation between gallbladder volume with BMI and age, whereas in the present study, to minimise the selection bias we selected the controls after doing matching for BMI and age as that of cases [30,31]. The grading of autonomic neuropathy did not vary much between type I and type II diabetes mellitus and so the gallbladder volume and contraction index did not show statistically significant difference between type I and type II diabetic patients. In the present study, the gallbladder contraction index is lowest among diabetic patients with longer duration compared to diabetic patients with lesser duration, which is because the neuropathy changes becomes more prevalent among long lasting diabetes and it was at par with the studies done by Agarwal AK et al., Singh S et al and Shaw ST et al., [18,19,32].

## Limitation(s)

Sample size was considered to be the limitation of the present study.

## CONCLUSION(S)

Ultrasonography is a very important imaging technique with advantages of being cost-effective and without any radiation side-effects and it was shown to be very useful in the evaluation of gallbladder changes in patients with type II DM. This study reveals that significant number of patients with type II DM along with autonomic neuropathy has gallbladder abnormalities in the form of increased gallbladder volume and reduced gallbladder contraction

index. Hence, it is recommended that all diabetic patients with symptoms of autonomic neuropathy should be evaluated for the fasting gallbladder volumes, postprandial gallbladder emptying, and gallbladder sludging; as these being the markers for progression to overt gall stone disease. Prospective studies with larger sample size should be carried out to further establish these results.

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