CHAPTER 5

CLINICAL INVESTIGATION BASED RESULTS

5.1 Oral Glucose Tolerance Test based clinical study over healthy non-diabetic subjects:

5.1.1 Introduction:

The present section represents the potential application of amplitude-modulated ultrasound and infrared light for noninvasive blood glucose measurement over healthy non-diabetic subjects, through OGTT (Oral Glucose Tolerance Test) based investigations. The sensitivity of our prototype for noninvasive blood glucose level measurement with respective to the glucose dose induced change in physiological glucose concentration has been reported here.

The consistent classifications when the OGTT is used are as follows:

- After 2 hours of 75 gm glucose dose intake, the blood glucose level below 140 mg/dl indicates normal glucose tolerance [ADA (2014)].
- After 2 hours of 75 gm dose glucose intake, the blood glucose level between 141 mg/dl to 199 mg/dl indicates Impaired Glucose Tolerance (IGT) [ADA (2014)].
- After 2 hours of 75 gm dose glucose intake, the blood glucose above 200 mg/dl conditionally diagnoses Diabetes Mellitus (DM) [ADA (2014)].

5.1.2 Study subjects:

In total five healthy adult subjects (three males and two females) participated in this clinical study. All the five study subjects are healthy, normal (age = 28±07 years, height = 168±4.0 cm, weight = 65.4±1.5 kg, and, Random Blood Glucose Level = 88±10.0 mg/dl) human beings. The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.1.3 Experimental protocol:

The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals, to measure blood glucose every 15 minutes up to an entire period of 2 hours respectively. The experimental steps followed for carrying out OGTT are as follows:

The trials were conducted in the morning and the subjects were instructed to fast (water is allowed) for 8–12 hours prior to the tests.

Step A. Fasting blood glucose level (zero time as base level for blood samples) of the study subjects has been measured at 0 min by our noninvasive blood glucose measurement unit (based on amplitude modulated ultrasound and infrared light) and by an established invasive glucometer (Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany).

Step B. The solution of 75 gm glucose (dextrose) in 100 ml of water, provided to all the subjects for drinking within a 5-minute period after step A.

Step C. This stage involves the acquisition of predicted (noninvasive) and reference (invasive) blood glucose readings at every 15 minutes up to a total period of 2 hours respectively.

Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.1.4 Blood glucose measurement:

During each experiment, for analysis purpose, acquisition of signal data occurs. The signal processing toolbox of MATLAB executes all the noninvasive signal data processing, and analyses peak amplitude (mV) in the Fast Fourier Transform (FFT) domain. These peak amplitude (mV) values in the FFT domain acts as the key function indicator for predicting blood glucose levels in human subjects. Based on this concept, calibration based look-up table (provided in Appendix-III) prepared for converting peak amplitude (mV) in FFT domain to its corresponding Predicted (Noninvasive) Blood Glucose Levels (mg/dl).

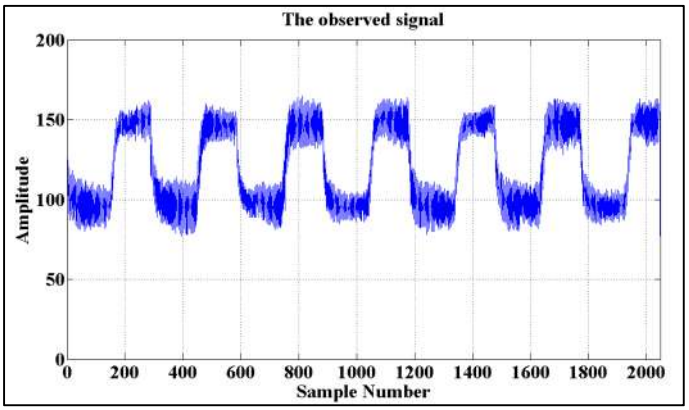
Further, in this present work, for cross validation, the Accu-Chek Active blood glucose monitoring device of Roche Diagnostics GmbH, Mannheim, Germany, measures the Reference (Invasive) blood glucose levels of the human subjects.

5.1.5 Result and Discussion:

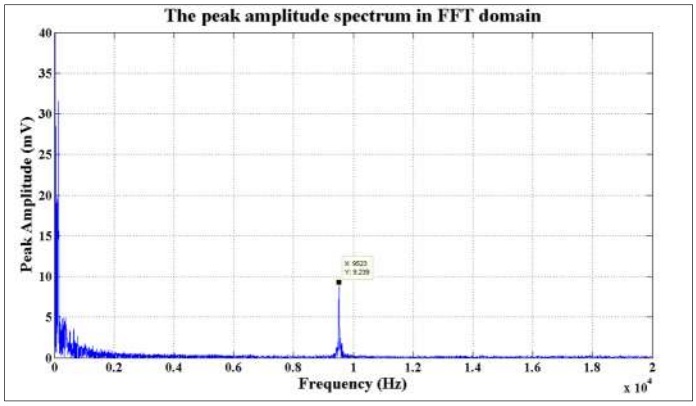
This clinical study depicts potential use of amplitude-modulated ultrasound with infrared technique for effective noninvasive blood glucose measurement.

The figures 5.1 to 5.3 depicts one of the sample noninvasive observed signals of study subject 1 and its respective spectrums in FFT domain, as acquired during 0 min (base time), 60 min, and 120 min of the OGTT respectively. The subject 01 OGTT test

based 0 min (base time) plot in FFT spectrum as depicted in figure 5.1(b), shows the peak amplitude of 9.23 mV (by noninvasive method) in correlation with invasive blood glucose level of 92 mg/dl. Again, the 60 min plot in figure 5.2(b) of subject 1 OGTT test reveals respective increase in peak amplitude from 9.23 mV to 13.02 mV (by noninvasive method) in FFT domain with corresponding increase in invasive blood glucose level from 92 mg/dl to 130 mg/dl. Consequently, the plot in figure 5.3(b) of subject 1 OGTT test at 120 min reveals corresponding decrease in peak amplitude from 13.02 mV to 8.52 mV (by noninvasive method) in FFT domain with response to decrease in invasive blood glucose level from 130 mg/dl to 85 mg/dl respectively.

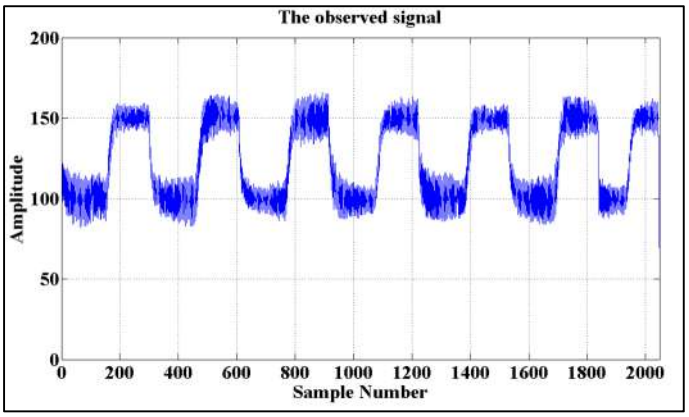


(a)

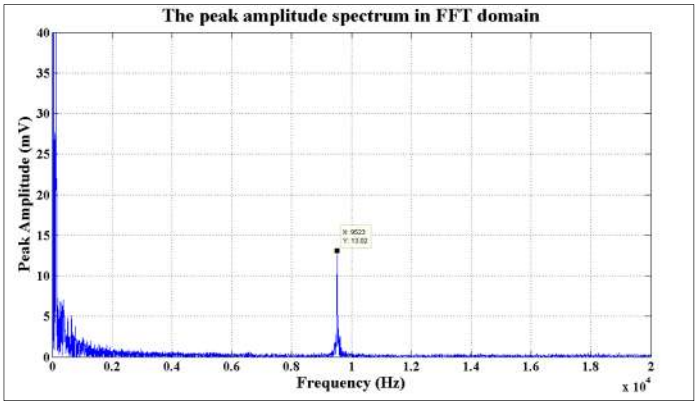


(b)

Figure 5.1(a) The observed signal as acquired from the subject 1 at 0 min and **(b)** its corresponding peak amplitude spectrum in the FFT domain

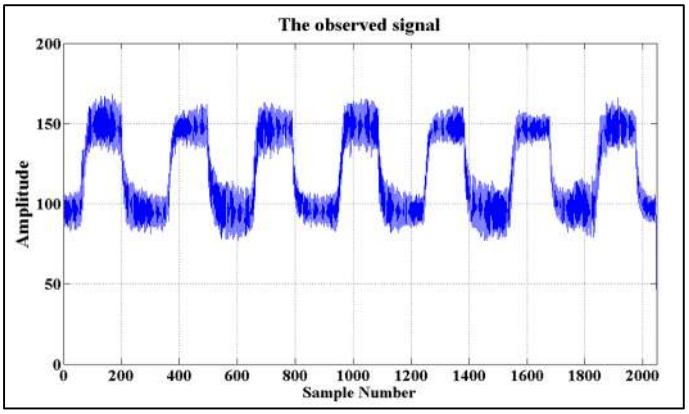


(a)

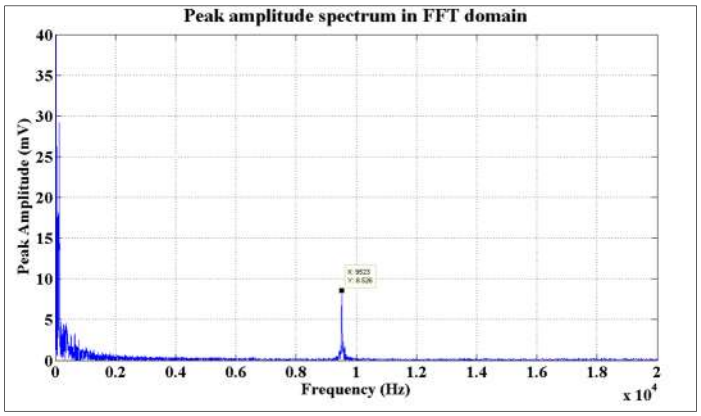


(b)

Figure 5.2 (a) The observed signal as acquired from the subject 1 at 60 min and **(b)** its corresponding peak amplitude spectrum in the FFT domain



(a)



(b)

Figure 5.3 (a) The observed signal as acquired from the subject 1 at 120 min and **(b)** its corresponding peak amplitude spectrum in the FFT domain

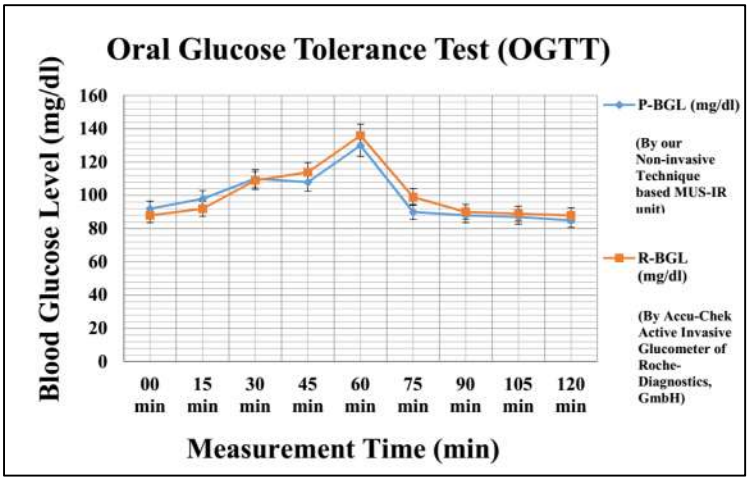


Figure 5.4: OGTT based time dependent sequential variations in the blood glucose levels; error bars indicate ±5 percentage error.

The figure 5.4 depicts the time sequence relationship between the Reference (Invasive) blood glucose readings and the Predicted (Noninvasive) blood glucose readings as acquired during OGTT screening test of the subject 1 respectively. The figure 5.4 shows good correlation between the invasive and noninvasive blood glucose levels of subject 1 during the OGTT based investigations. The error bars show the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

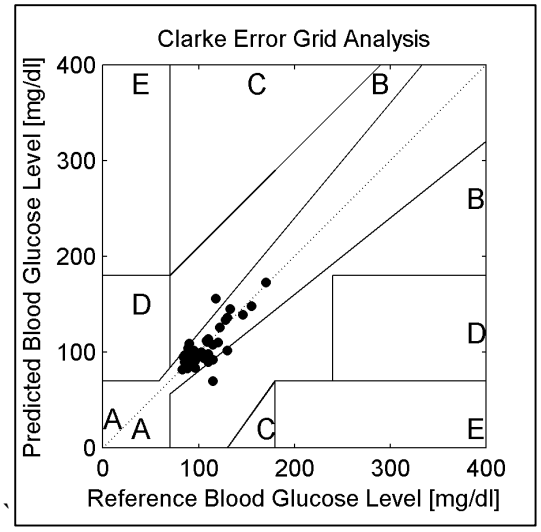


Figure 5.5: Clarke Error Grid Analysis plot as obtained from the OGTT investigation.

Now, the figure 5.5 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during OGTT based clinical investigation over five healthy study subjects. In Table 5.1, the Clarke Error Grid analysis shows the percentage of the total data pairs (45) falling in the zones A, B, C, D, and E are 91.12% (41 data pairs), 08.88% (04 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 45 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.1: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels as acquired during OGTT over healthy subjects.

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	41	04	00	00	00
Percentage of total data pairs occupying A to E zones	91.12%	08.88%	00.00%	00.00%	00.00%

Table 5.2: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	Our assessment based values	Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data	
		Published data ranges	References
MAE	09.00 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	05.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	12.83 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	08.43 %	(08.60 to 40.80)[d] %	
%MdARE	05.55 %	(07.70 to 30.00)[e] %	
SEP	12.87 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.80	(00.49 to 00.95)[g]	

The Table 5.2 depicts our performance assessment values as acquired during OGTT over five healthy study subjects and results comparison with other Noninvasive

Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 09.00 mg/dl, 05.00 mg/dl, and 12.83 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 08.43%, and 05.55% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 12.87 mg/dl and 00.80 respectively. Further, as depicted from Table 5.2, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring System(s). Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.1.6 Conclusion:

The OGTT based results of our clinical study performed over the human subject's shows the medically significant relationship in between the Predicted Blood Glucose Levels and Reference Blood Glucose Levels. Further, Clarke Error Grid Analysis and statistical analysis yields promising results. All this aspects proves the efficiency our noninvasive technique based prototype unit in performing medically significant noninvasive blood glucose measurement in the healthy human subjects.

5.2 The effect of different glucose concentrations over blood glucose levels:

5.2.1 Introduction:

This section evaluates the efficiency of our noninvasive technique based prototype to measure the different glucose concentration (25 gm/100 ml, 50 gm/100 ml, and 75 gm/100 ml) induced blood glucose level changes in the study subjects. In this present work, the clinical study continued for three days to observe the effect of each aqueous glucose concentration solution over the study subject's blood glucose levels.

5.2.2 Study subjects:

In total ten adult subjects (eight males and two females) participated in this clinical study. All the study subjects are healthy, normal (age = 25.5±3.5 years, height =

161±4.0 cm, weight = 72±7.0 kg, and, Random Blood Glucose Level = 85±5.0 mg/dl) human beings. The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.2.3 Experimental protocol:

The clinical study held in the morning and the subjects were instructed to fast (water is allowed) for 10 to 12 hours prior to the tests.

Step A. The noninvasive and invasive fasting blood glucose level of the human subjects were acquired at 0 min (base time readings) respectively.

Step B. The solution of 25 gm glucose (dextrose) in 100 ml of water, provided to all the subjects for drinking within a 5-minute period after step A.

Step C. This phase involves acquisition of predicted (noninvasive) and reference (invasive) blood glucose readings from all the 10 subjects after 1 hour and 2 hour of glucose solution intake respectively.

The same protocol followed for observing 50 gm/100 ml and 75 gm/100 ml aqueous glucose solutions (w/v) effect over 10 healthy subject's blood glucose levels for next 02 days respectively. The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals during their fasting stages, and one-hour, two hour after respective glucose solution intakes during abovementioned occasions.

Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.2.4 Blood glucose measurement:

In this present work, our noninvasive technique based prototype unit performs the predicted (noninvasive) blood glucose measurements. Similarly, for cross validation, the Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany measures the reference (invasive) blood glucose levels of the human subjects.

5.2.5 Result and Discussion:

This clinical study demonstrates the potential aspect of our noninvasive technique in detecting various glucose concentration induced change in the blood glucose levels.

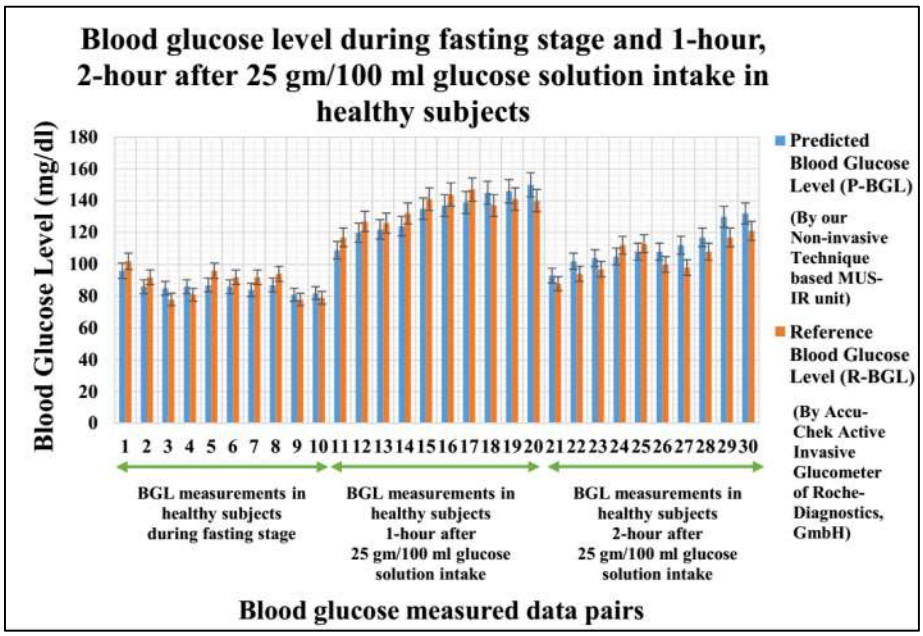


Figure 5.6: The effect of 25 gm/100 ml glucose solution (w/v) on the fasting BGL; error bars indicate ±5 percentage error.

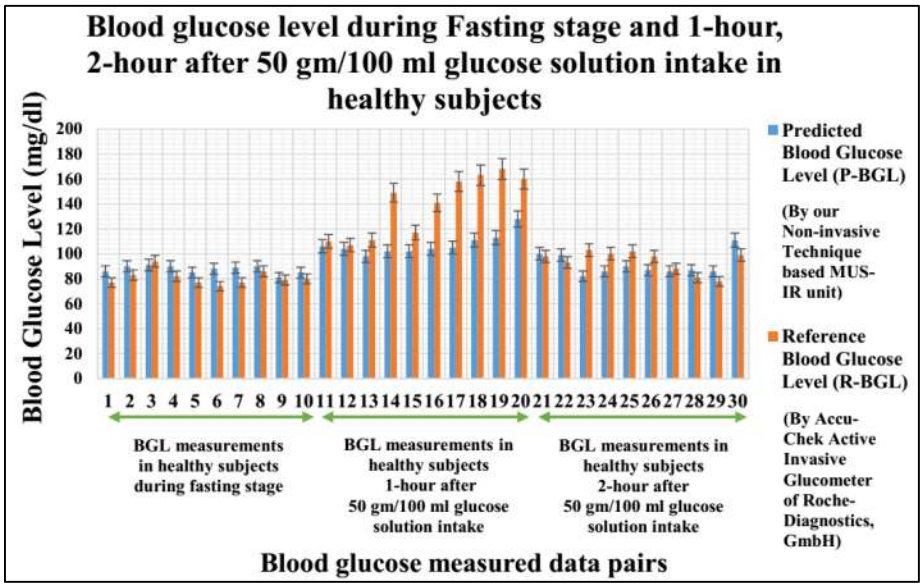


Figure 5.7: The effect of 50 gm/100 ml glucose solution (w/v) on the fasting BGL; error bars indicate ±5 percentage error.

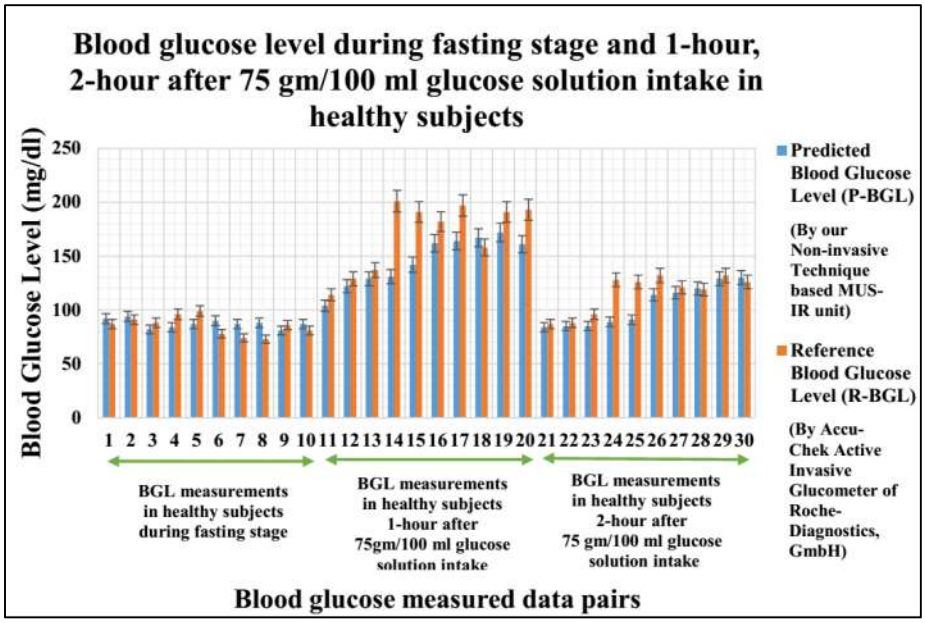


Figure 5.8: The effect of 75 gm/100 ml glucose solution (w/v) on the fasting BGL; error bars indicate ±5 percentage error

The figures 5.6, 5.7, and 5.8 depict the effect of 25 gm/100 ml, 50 gm/100 ml, and 75 gm/100 ml aqueous glucose solutions effect over the blood glucose levels. The pattern of those values as seen from the figures 5.6, 5.7, and 5.8 indicates that with increase in glucose concentration, the blood glucose increases from that of the fasting stage and reaches the peak levels after one hour of glucose intake. However, after 2 hour of glucose intake, the respective healthy subject's blood glucose levels are in normal ranges as balanced by the individual body's physiological (blood glucose regulation) mechanisms respectively. Further, the figure 5.6, 5.7, and 5.8 reveals that blood glucose level shoots maximum during 75 gm/100 ml aqueous glucose solution intake in compared to 50 gm/100 ml and 25 gm/100 ml aqueous glucose solution intakes respectively. Simultaneously, in this present work, the figures 5.6, 5.7, 5.8 depict noninvasive and invasive blood glucose measurements performed to evaluate the working efficiency of our indigenously developed noninvasive technique based prototype unit. The error bars show the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

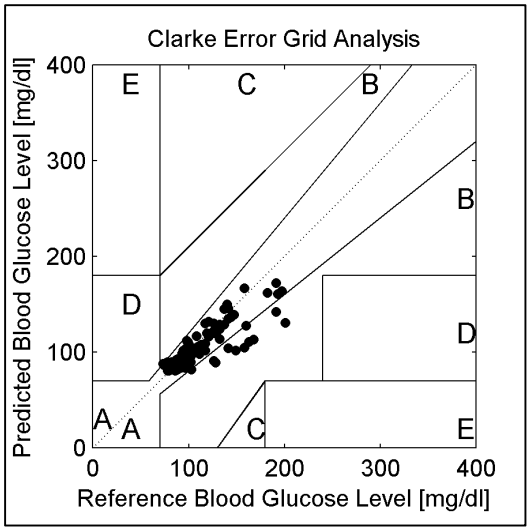


Figure 5.9: Clarke Error Grid Analysis

Now, the figure 5.9 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over ten healthy study subjects. In Table 5.3, the Clarke Error Grid analysis shows the percentage of the total data pairs (90) falling in the zones A, B, C, D, and E are 87.78% (79 data pairs), 12.22% (11 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 90 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.3: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	79	11	00	00	00
Percentage of total data pairs occupying A to E zones	87.78%	12.22%	00.00%	00.00%	00.00%

Table 5.4: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	Our assessment based values	Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data	
		Published data ranges	References
MAE	12.92 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin.(2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	08.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	18.81 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	10.52 %	(08.60 to 40.80)[d] %	
%MdARE	07.72 %	(07.70 to 30.00)[e] %	
SEP	12.58 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.84	(00.49 to 00.95)[g]	

The Table 5.4 depicts our performance assessment values as acquired during this clinical investigation over ten healthy study subjects and results comparison with other

Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 12.92 mg/dl, 08.00 mg/dl, and 18.81 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 10.52%, and 07.72% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 12.58 mg/dl and 00.84 respectively. Further, as depicted from Table 5.4, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.2.6 Conclusion:

In this section, our clinical investigation indicate that blood glucose levels increase with increase in glucose concentration in all the human study subjects. At the same time, the individual's body glucose homeostasis-mechanism regulates and controls the blood glucose levels. Further, the Clarke Error Grid and statistical analysis yields promising results that prove the efficiency of our noninvasive technique based prototype unit in detecting different glucose concentration induced respective change in blood glucose levels.

5.3 Study over pre-diabetic subjects:

5.3.1 Introduction:

This section evaluates the efficiency for our noninvasive technique based prototype to measure the blood glucose concentration as obtained from the normal healthy and pre-diabetic human subjects. This clinical study performed into two phases. Phase I (1st day) includes blood glucose measurement during fasting stage and 2 hour after meal intake. Phase II (2nd day) includes blood glucose measurement during fasting stage and 2 hour after 75 gm/100 ml aqueous glucose solution intake.

5.3.2 Glucose sensing in pre-diabetics:

Usually, pre-diabetics suffer from Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG) symptoms in their daily lifestyle. In general, the physicians [IDF (2013); ADA (2014)] distinguishes this phenomenon as the transitional group of peoples whose blood glucose levels, even though not matches the criterion for diabetes, and are however too elevated to judge it as normal. Such groups are characterized as possessing Fasting Plasma Glucose concentration above or equal to 100 mg/dl but lesser than 126 mg/dl respectively. Again, 2 hour after their OGTT (Oral Glucose Tolerance Test), the blood glucose values exists above 140 mg/dl but lesser than 200 mg/dl respectively. Both the IFG and IGT belong to the transitional phases in any glycemic disorder related pathological stages [ADA (2014)].

5.3.3 Study subjects:

In total ten adult subjects (seven males and three females, age = 35±6.5 years, height = 173±5.5 cm, weight = 70±11.5 kg) participated in this clinical study. The Two study subjects are healthy normal human beings. Other eight adult subjects are with the history of pre-diabetic symptoms like IGT and IFG respectively. The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.3.4 Experimental protocol:

The clinical study conducted in two phases.

The Phase I (1st day) includes determination of reference (invasive) and predicted (noninvasive) blood glucose values of both the healthy normal and pre-diabetic subjects after overnight fasting and after 02 hours of meal intake respectively.

The Phase II (2nd day) includes determination of (invasive) reference and predicted (noninvasive) blood glucose values of both the healthy normal and pre-diabetic subjects next day after overnight fasting and 2 hours after 75 gm/100 ml of aqueous glucose solution (w/v) intake respectively.

The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals respectively.

Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.3.5 Blood glucose measurement:

In this present work, our noninvasive technique based prototype unit performs the predicted (noninvasive) blood glucose measurements. Similarly, for cross validation, the Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany measures the reference (invasive) blood glucose levels of the human subjects.

5.3.6 Result and Discussion:

This clinical study demonstrates the potential aspect of our noninvasive technique in detecting blood glucose levels in healthy and pre-diabetic subjects. The figures 5.10 and 5.11 shows the pattern of reference (invasive) and predicted (noninvasive) blood glucose levels as obtained from the healthy normal and pre-diabetic subjects during Phase I and II experimental clinical studies respectively.

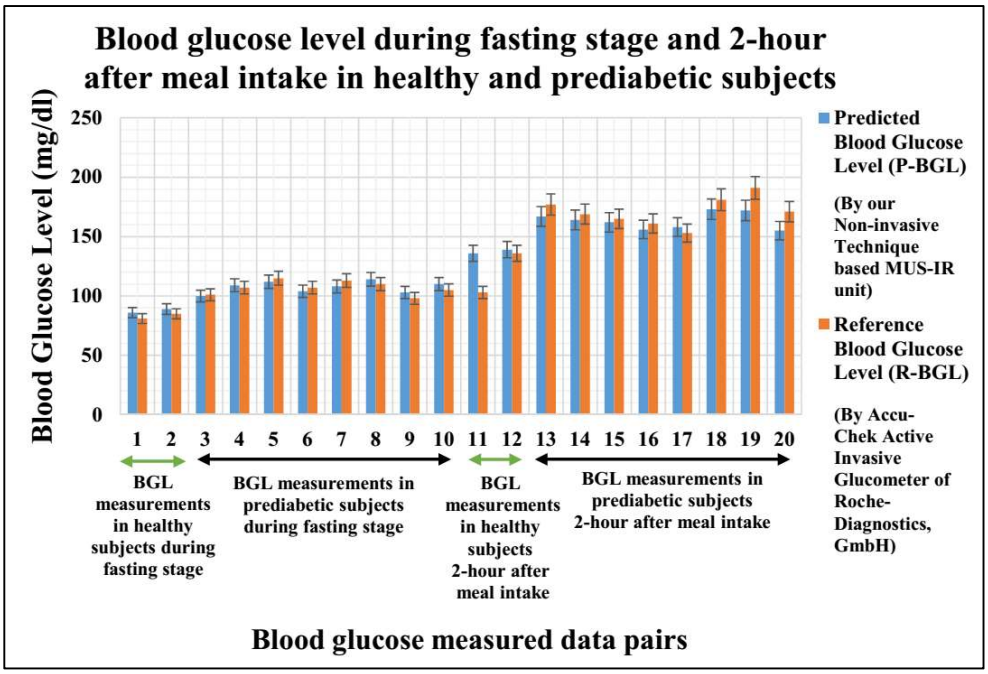


Figure 5.10: BGL after meal intake in healthy and pre-diabetic subjects; error bars indicate ±5 percentage error.

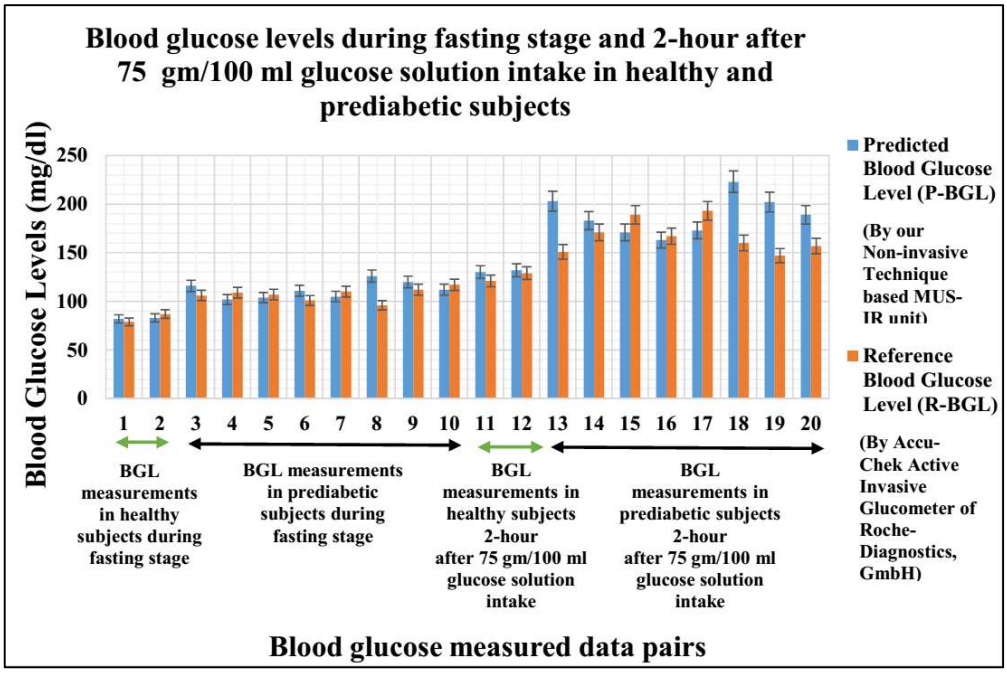


Figure 5.11: BGL after 75 gm/100 ml glucose intake in healthy and pre-diabetic subjects; error bars indicate ±5 percentage error.

Both the figures 5.10 and 5.11 depict that fasting blood glucose levels of the normal subject's exists below 100 mg/dl. Again, 2 hour after meal intake or 75 gm/100 aqueous glucose solution intake the blood glucose levels of healthy subjects exists below 140 mg/dl respectively. Similarly, both the figures 5.10 and 5.11 depict that fasting blood glucose levels in majority of the pre-diabetic subjects exists equal to or above 100 mg/dl but lower than 126 mg/dl. Again, 2 hour after meal intake or 75 gm/100 aqueous glucose solution intake the majority of pre-diabetic subject's blood glucose level exists equal to or above 140 mg/dl but less than 200 mg/dl respectively. However, few pre-diabetic subject's blood glucose levels marginally crosses fasting blood glucose level of 126 mg/dl [ADA 2014] and 200 mg/dl [ADA 2014] level mark even after 2 hour of meal intake or 75 gm/100 ml aqueous glucose solution intake, which indicates that these subjects are more prone to be diabetic in near future. Simultaneously, in this present work, the figures 5.10, 5.11 depict noninvasive and invasive blood glucose measurements performed to evaluate the working efficiency of our indigenously developed noninvasive technique based prototype unit. The error bars show the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for

further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

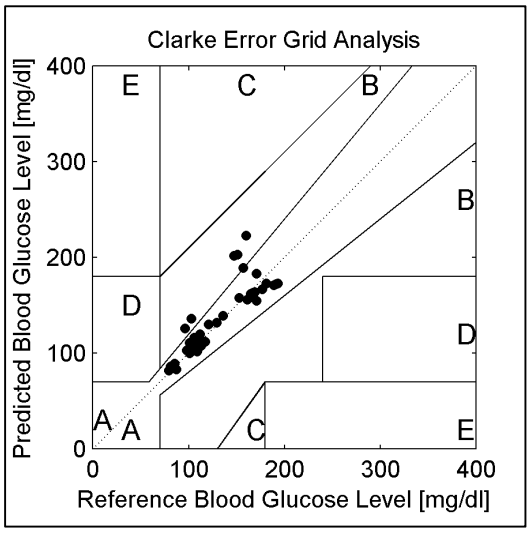


Figure 5.12: Clarke Error Grid Analysis

Now, the figure 5.12 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over two healthy normal and eight pre-diabetic study subjects. In Table 5.5, the Clarke Error Grid analysis shows the percentage of the total data pairs (40) falling in the zones A, B, C, D, and E are 85.00% (34 data pairs), 15.00% (06 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 40 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.5: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	34	06	00	00	00
Percentage of total data pairs occupying A to E zones	85.00%	15.00%	00.00%	00.00%	00.00%

Table 5.6: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	12.42 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	05.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	19.43 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	09.16 %	(08.60 to 40.80)[d] %	
%MdARE	04.73 %	(07.70 to 30.00)[e] %	
SEP	19.04 mg/dl	(10.00 to 54.00)[f] mg/dl	
r-value	00.86	(00.49 to 00.95)[g]	

The Table 5.6 depicts our performance assessment values as acquired during this clinical investigation over two healthy normal and eight pre-diabetic study subjects.

Further, the Table 5.6 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 12.42 mg/dl, 05.00 mg/dl, and 19.43 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 09.16%, and 04.73% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 19.04 mg/dl and 00.86 respectively.

Further, as depicted from Table 5.6, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.3.7 Conclusion:

The reference (invasive) and predicted (noninvasive) blood glucose levels of all the subjects (healthy normal and pre-diabetics) occupies medically significant and acceptable A and B zones in the Clarke Error Grid Analysis. None of the BGL readings occupies medically insignificant and potentially dangerous C to E zones in the Clarke Error Grid plot. Further, statistical analysis provides significant and promising results. Therefore, our noninvasive technique based prototype (MUS-IR) unit has been successful in measuring blood glucose levels in healthy normal and pre-diabetic subjects respectively.

5.4 Clinical study over Diabetic subjects:

5.4.1 Introduction:

This clinical study monitors the blood glucose level (both invasive and noninvasive) in diabetic subjects during fasting, postprandial, and random stages to evaluate the performance of our noninvasive technique based prototype (MUS-IR) unit.

5.4.2 Blood glucose supervision:

Blood glucose monitoring refers to the technique of measuring glucose concentration levels in the blood. Principally, it is essential in diabetes mellitus management and care [IDF (2013); ADA (2013); Watkins (2003)]. This type of testing helps in prompt management of high (hyperglycemia) or very low (hypoglycemia) blood glucose levels respectively [ADA (2013); Watkins (2003)].

In this present work, Fasting blood glucose stage refers to the Blood Glucose Level after 8 to 10 hours of overnight fasting; only water allowed. Postprandial blood glucose stage refers to the Blood Glucose Level 2 hour after meal intake. Random blood glucose stage refers to Blood Glucose Level at any time during daytime in non-fasting subjects [Khalil (2009); Tuchin (2009); Lam (2008)].

5.4.3 Study subjects:

In total thirty-four adult subjects participated in this clinical study. Out of which, three of them were in normal health conditions (two male, one female, aged 26.5±3.5 years, of height 175±3.0cm, weight 76.2±11 kg) and took no medications. The other 31 subjects were all Diabetic subjects (19 males, 12 females, and aged 45.5±4.0 years, of height 170±2.0cm, weight 74.5±9 kg). Eight subjects had Type I Diabetes and twenty-three subjects had Type II Diabetes.

The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.4.4 Experimental protocol:

This clinical study monitors the diabetic subjects fasting, postprandial, and random stages to evaluate the performance of our noninvasive technique based prototype (MUS-IR) unit in measuring blood glucose levels.

The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals respectively.

Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.4.5 Blood glucose measurement:

In this present work, our noninvasive technique based prototype unit performs the predicted (noninvasive) blood glucose measurements. Similarly, for cross validation, the Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany measures the reference (invasive) blood glucose levels of the human subjects.

5.4.6 Result and Discussion:

The figures 5.13 to 5.15 depict the reference (invasive) and predicted (noninvasive) technique based blood glucose values as obtained during fasting, postprandial, and random stages of the healthy normal, Type I and Type II diabetic subjects.

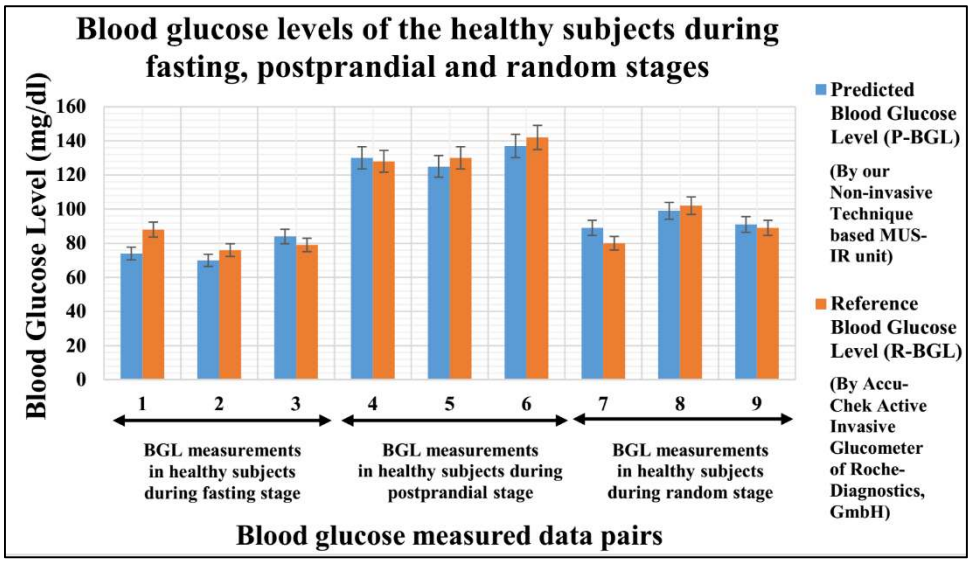


Figure 5.13: R-BGL (Invasive) and P-BGL (Noninvasive) blood glucose levels of the healthy subjects during Fasting, Postprandial, and Random stage; error bars indicate ±5 percentage error.

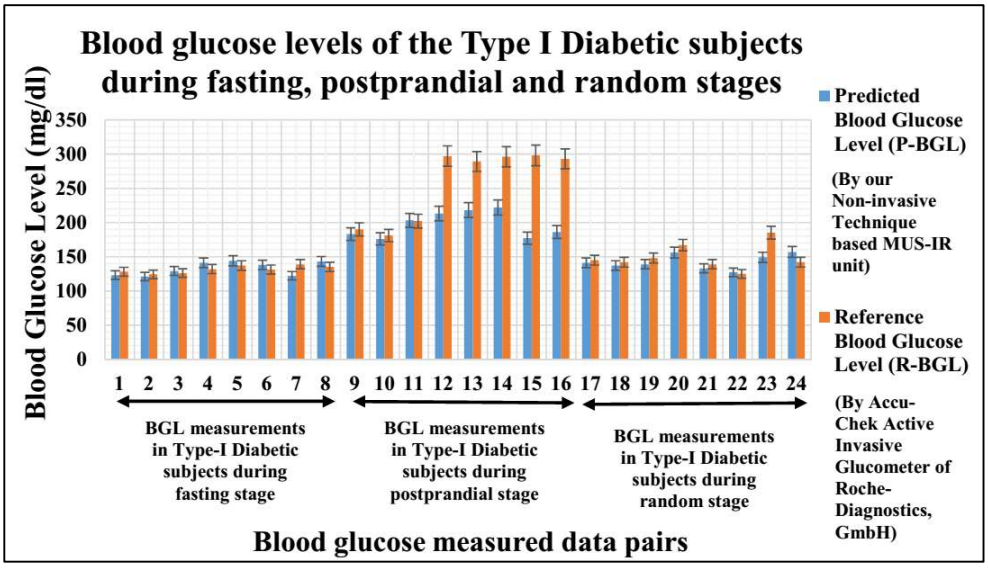


Figure 5.14: R-BGL (Invasive) and P-BGL (Noninvasive) blood glucose levels of the Type I Diabetic subjects during Fasting, Postprandial, and Random stage; error bars indicate ±5 percentage error.

The figure 5.13 depict that blood glucose levels of the healthy normal subjects during the course of fasting, postprandial, and random stages swings between 80 mg/dl to 140 mg/dl respectively.

However, both the figure 5.14 and 5.15 depicts that the blood glucose levels of the Type I and Type II diabetic subjects during the course of fasting, postprandial, and random stages are in much higher stages in comparison with normal subject's blood glucose levels respectively.

Proper blood glucose management and medications are essential to manage blood glucose levels in the diabetic subjects [ADA 2014].

The error bars in figures 5.13, 5.14 and 5.14 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

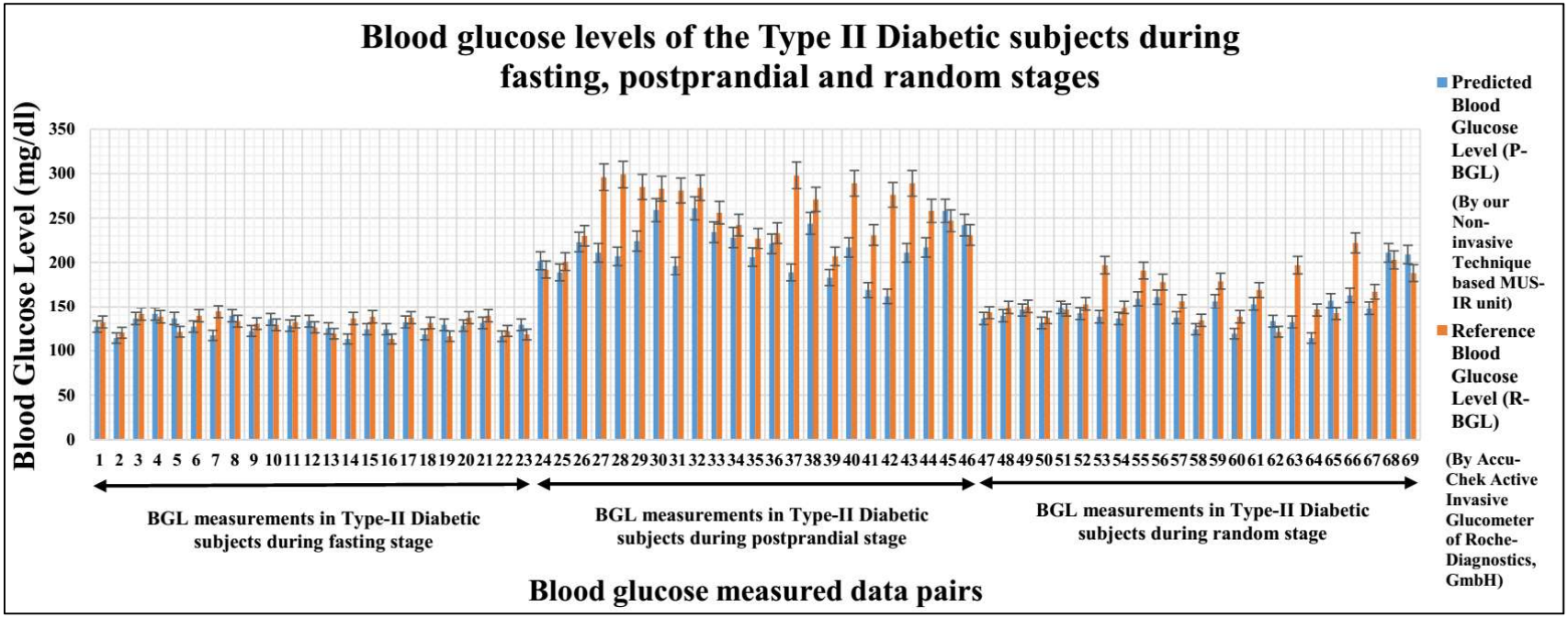


Figure 5.15: R-BGL (Invasive) and P-BGL (Noninvasive) blood glucose levels of the Type II Diabetic subjects during Fasting, Postprandial, and Random stages; error bars indicate ±5 percentage error.

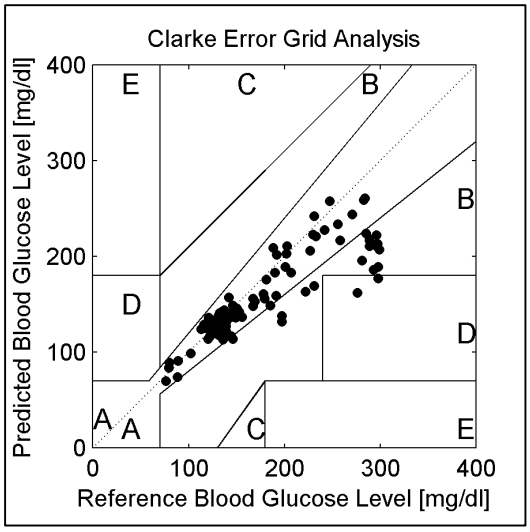


Figure 5.16: Clarke Error Grid Analysis

Now, the figure 5.16 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over three healthy normal, eight Type I Diabetic, and twenty-three Type II Diabetic study subjects. In Table 5.7, the Clarke Error Grid analysis shows the percentage of the total data pairs (102) falling in the zones A, B, C, D, and E are 82.35% (84 data pairs), 15.68% (16 data pairs), 00.00% (00 data pairs), 01.97% (02 data pairs), and 00.00% (00 data pairs), respectively. The Table 5.7 depicts that 100 out of total 102 data pair sets, exists between the medically acceptable A and B zones respectively. Only two data pair sets falls on the D zone. None of the data pair sets occupies medically insignificant and potentially dangerous C and E zones respectively.

Table 5.7: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	84	16	00	02	00
Percentage of total data pairs occupying A to E zones	82.35%	15.68%	00.00%	01.97%	00.00%

Table 5.8: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	23.41 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	11.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	36.89 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	11.16 %	(08.60 to 40.80)[d] %	
%MdARE	07.67 %	(07.70 to 30.00)[e] %	
SEP	20.64 mg/dl	(10.00 to 54.00)[f] mg/dl	
r- value	00.87	(00.49 to 00.95)[g]	

The Table 5.8 depicts our performance assessment values as acquired during this clinical investigation over three healthy normal, eight Type I Diabetic, and twenty-three

Type II Diabetic study subjects. Further, the Table 5.8 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 23.41 mg/dl, 11.00 mg/dl, and 36.89 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 11.16%, and 07.67% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 20.64 mg/dl and 00.87 respectively. Further, as depicted from Table 5.8, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.4.7 Conclusion:

In this present work, we observed that the blood glucose levels of diabetic subjects are elevated as compared to the normal subjects. Proper glycemic management is essential for diabetic subjects to avoid future medical emergencies. Further, our noninvasive prototype (MUS-IR) unit has performed blood glucose measurement in normal healthy, Type I and Type II diabetic subjects here. The results showed good sensitivity and positive aspect about the working efficiency of our prototype (MUS-IR) unit in determining blood glucose levels during fasting, postprandial, and random stages in study subjects respectively.

5.5 Five daily sessions of blood glucose measurement in a healthy normal and a diabetic subject:

5.5.1 Introduction:

This section represents reference (invasive) and predicted (noninvasive) blood glucose level monitoring over five daily sessions during fasting, postprandial and random stages of a healthy normal subject and a Type II diabetic subject respectively. A lab-based study performed to evaluate the sensitivity performance of our noninvasive

technique based prototype (MUS-IR) unit in continuous blood glucose monitoring for five days.

5.5.2 Study subjects:

In total two male subjects participated in this clinical study. The healthy normal subject age is around 28 years, height = 163 cm and, weight = 72 kg. The other Type II diabetic subjects age is around 35 years, height = 165 cm and, weight = 85 kg. The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.5.3 Experimental protocol:

In this present work, the blood glucose measurement performed for five consecutive days during fasting, postprandial, and random blood glucose levels of the healthy normal and Type II Diabetic subjects. This type of clinical study evaluates the efficiency of our noninvasive technique based prototype unit in blood glucose monitoring for five consecutive days. The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals respectively. Further, the Clarke Error Grid and Statistical analysis applied to measure the performance metrics of our noninvasive technique based prototype unit in regular monitoring of blood glucose levels for five consecutive days in human subjects.

5.5.4 Blood glucose measurement:

In this present work, our noninvasive technique based prototype unit performs the predicted (noninvasive) blood glucose measurements. Similarly, for cross validation, the Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany measures the reference (invasive) blood glucose levels in the human subjects.

5.5.5 Result and Discussion:

The five daily sessions of blood glucose detections by our noninvasive technique have shown us good sensitivity and potentiality. The figure 5.17 depicts the healthy normal subject invasive and noninvasive blood glucose readings as obtained throughout the five daily sessions of fasting, postprandial, and random stages. Similarly, the figure 5.18 depicts the Type II diabetic subject invasive and noninvasive blood glucose readings as obtained throughout the five daily sessions of fasting, postprandial, and random stages.

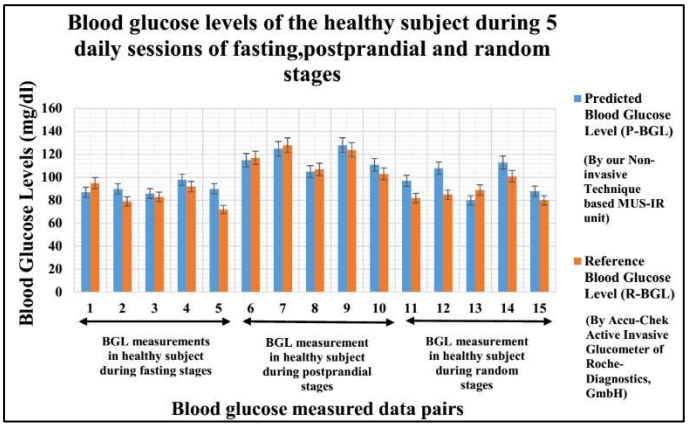


Figure 5.17: The Blood Glucose Level values as obtained by the Predicted (noninvasive) and Reference (invasive) methods from the normal subject during 5 daily sessions of fasting, postprandial and random stages; error bars indicate ±5 percentage error.

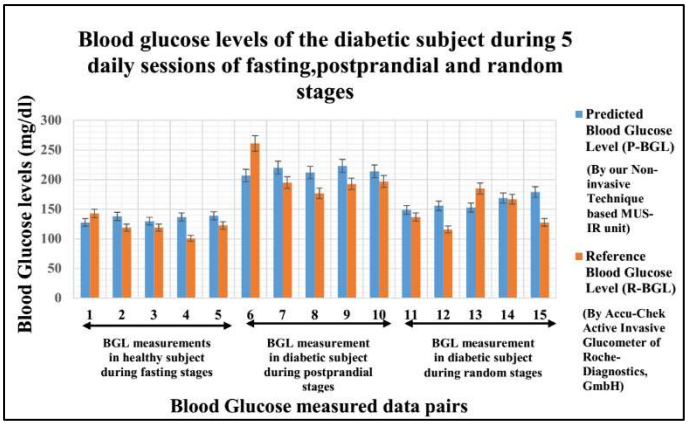


Figure 5.18: The Blood Glucose Level values as obtained by the Predicted (noninvasive) and Reference (invasive) methods from the Type II Diabetic subject during five daily sessions of fasting, postprandial, and random stages; error bars indicate ±5 percentage error.

Both the figures 5.17 and 5.18 depict good correlation between invasive and noninvasive blood glucose levels throughout the five daily sessions of fasting, postprandial, and random stages respectively. The error bars in figures 5.17 and 5.18 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

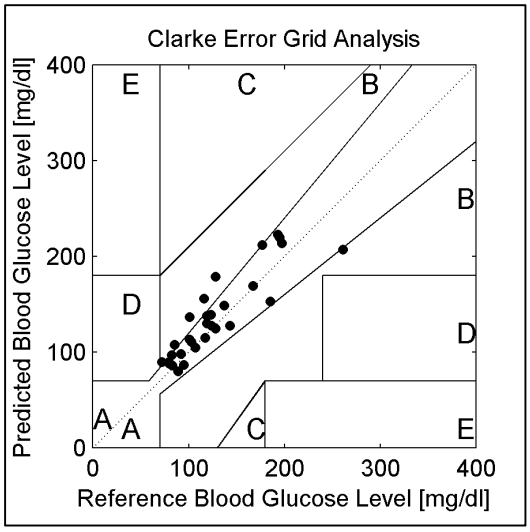


Figure 5.19: Depicts the Clarke Error Grid-based Analysis of the Blood Glucose Level values as obtained from both the Subjects (Normal and Type II Diabetic) during 5 daily sessions of Fasting, Postprandial and Random stages.

Now, the figure 5.19 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over one healthy normal, and one Type II Diabetic study subject respectively. In Table 5.9, the Clarke Error Grid analysis shows the percentage of the total data pairs (30) falling in the zones A, B, C, D, and E are 80.00% (24 data pairs), 20.00% (06 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 30 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.9: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	24	06	00	00	00
Percentage of total data pairs occupying A to E zones	80.00%	20.00%	00.00%	00.00%	00.00%

Table 5.10: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	17.56 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	13.50 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	22.56 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	13.83 %	(08.60 to 40.80)[d] %	
%MdARE	11.18 %	(07.70 to 30.00)[e] %	
SEP	20.44 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.89	(00.49 to 00.95)[g]	

The Table 5.10 depicts our performance assessment values as acquired during this clinical investigation over one healthy normal and one Type II Diabetic study subjects.

Further, the Table 5.10 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 17.56 mg/dl, 13.50 mg/dl, and 22.56 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 13.83%, and 11.18% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 20.44 mg/dl and 00.89 respectively. Further, as depicted from Table 5.10, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.5.6 Conclusion:

We have shown the sensitivity and working efficiency of our prototype (MUS-IR) unit for noninvasive blood glucose determinations in healthy normal and Type II Diabetic subjects for five days. In addition, this study allows us to follow the subject's intra day-to-day behavior, variability, and trends. The Clarke Error Grid-based performance analysis depicts that all the blood glucose readings occupy medically significant and acceptable A to B zones. Moreover, none of the estimated readings occupies medically insignificant and potentially dangerous C to E zones respectively. Statistical analysis yields promising results. Hence, all the data indicates towards the acceptable working sensitivity, efficiency, and potentiality of our prototype (MUS-IR) unit for noninvasive blood glucose determination in human subjects.

5.6 Blood Glucose and Glycated Hemoglobin relationship:

5.6.1 Introduction:

This section represents the clinical study about the fasting blood glucose measurement in the healthy normal and diabetic subjects by reference (invasive) and indigenously developed predicted (noninvasive) technology. Further, this section includes

the correlation between the fasting blood glucose (glycemic index) with the Glycated hemoglobin (HbA1c) biomarker profile of both the normal healthy and diabetic subjects. A lab-based study performed to evaluate the sensitivity performance of our noninvasive technique based prototype (MUS-IR) unit in measuring fasting blood glucose levels in healthy normal and diabetic subjects.

The Table 5.11 as per ADA (2014) guidelines depicts the HbA1c levels and corresponding blood glucose levels in healthy, pre-diabetic, and diabetic subjects.

Table 5.11: Blood Glucose Levels and corresponding HbA1c levels [ADA (2014)]

Subjects	Percentage of HbA1c	Fasting Plasma Glucose		Oral Glucose Tolerance Test	
		mg/dl	mmol/l	mg/dl	mmol/l
Diabetic	6.5 and above	126 and above	7 and above	200 and above	11.1 and above
Prediabetic	5.7 to 6.4	100 to 126	5.56 to 7	140 to 199	7.77 to 11
Healthy	below 5.8	99 and below	3.89 and 5.5	139 and below	7.72 and below

5.6.2 Study subjects:

In total twenty (thirteen males, seven females) adult subjects participated in this clinical study. Ten subjects are healthy normal (seven males, three females, age = 26.6±2.0 years, height = 176±4.2 cm, weight = 74.6±12 kg). Other, ten subjects had diabetes (six males, four females, age = 34±5.5 years, height = 172±4.5 cm, weight = 69±13.5 kg). The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.6.3 Experimental protocol:

All the study subjects followed their normal physical exercises and food habits before five days of the clinical study. The clinical study held in the morning and the subjects were instructed to fast (water is allowed) for 10 to 12 hours prior to the tests. The bio-specimen in the form of blood samples collected from the left hand for fasting

invasive blood glucose and Glycated hemoglobin level measurements. Simultaneously, the right hand index finger of the study subjects serves as the measurement site for blood glucose measurement by our noninvasive technique based prototype unit. The error bars in figures 5.20, 5.21 and 5.22 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here. Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.6.4 Invasive and Noninvasive determination of fasting blood glucose levels:

For noninvasive fasting blood glucose determination, we have utilized our indigenously developed noninvasive technique based prototype (MUS-IR) unit. For cross validation, the Accu-Chek Active of Roche diagnostics GmbH, Mannheim, Germany determines the invasive fasting blood glucose levels.

5.6.5 Glycated Hemoglobin (HbA1c) levels measurement:

Ion Exchange Resin method is the standard test methodology for HbA1c measurement. For Glycated hemoglobin estimations, first we have performed in-vitro quantitative glycosylated hemoglobin measurement in human blood by utilizing Ion Exchange Resin method. During column chromatography, the fast fraction glycosylated hemoglobin like HbA1a, HbA1b and HbA1c elute first in the columns due to lower isoelectric points. The bulk hemoglobin portion obtained known as the HbAo, which is non-glycosylated in nature. The next step includes whole blood hemolysed sample preparations, application over the loosely binding cation exchange resins beds. During preparation of whole blood hemolysate samples and binding processes, the expulsion of labile fractions occurs. Now, during the mixing process, HbAo binds actively with the ion exchange beds leaving behind the GHb portion as a supernatant fraction. Supernatant fractions rich in GHb portions were collected using filter separator after the mixing process. The Digital Spectrometer Model 305 of M.S Electronics Pvt. Ltd., (India), utilized in this present work to measure absorbance of the Glycosylated Hemoglobin (GHb) fraction and the Total Hemoglobin (THb) fraction at 415 nm to provide the percentage of glycosylated hemoglobin. Subsequently, the absorbance ratio of the

Glycosylated hemoglobin and the Total hemoglobin fraction of the Control and the Test measured to estimate the percentage of Glycosylated hemoglobin in the samples. The range between (04.00%-20.0%) the Glycosylated hemoglobin procedure shows linearity. Secondly, after this stage, the standard conversion Tables for percentage of GHb to percentage of HbA1c values followed to obtain the percentage of HbA1c values respectively [Nathan *et al.* (1984); Bunn *et al.* (1975); Bates *et al.* (1978); Trivelli *et al.* (1971)].

5.6.6 Result and Discussion:

The Table 5.12 and figure 5.20 depicts the medically significant and acceptable correlation between the invasive, noninvasive fasting blood glucose readings as obtained from the healthy (1 to 10) study subjects during our clinical study. Further, herein as per conversion chart of GHb% to HbA1c% [Nathan *et al.* (1984); Bunn *et al.* (1975); Bates *et al.* (1978); Trivelli *et al.* (1971)], all the GHb% values are converted to HbA1c% values.

Table 5.12: Reference (Invasive), Predicted (Noninvasive) fasting blood glucose values and its corresponding GHb%, and HbA_{1c}% values as obtained from the healthy subjects

Healthy Normal Subjects	Blood Glucose Level (mg/dl)		GHb% values	HbA1c% values
	Fasting stage			
	R-BGL (by Invasive method)	P-BGL (by Noninvasive method)		
Subject 1	86	99	7.0	5.13
Subject 2	76	90	6.7	4.88
Subject 3	86	83	7.0	5.13
Subject 4	71	88	6.5	4.71
Subject 5	87	84	7.1	5.22
Subject 6	95	91	7.4	5.47
Subject 7	88	89	7.1	5.22
Subject 8	90	87	7.2	5.30
Subject 9	92	88	7.3	5.39
Subject 10	105	81	7.7	5.72

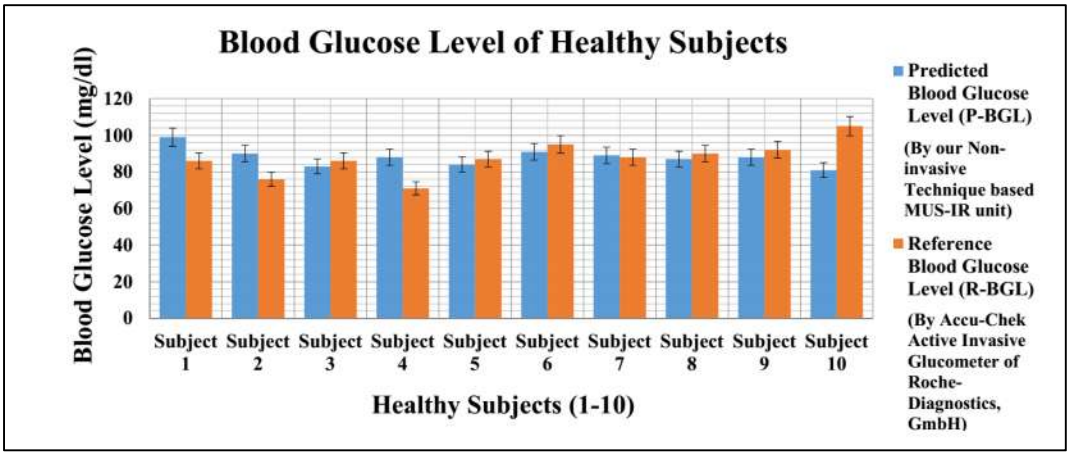


Figure 5.20: Reference (Invasive), Predicted (Noninvasive) fasting blood glucose values as obtained from the healthy subjects; error bars indicate ±5 percentage error.

Table 5.13: Reference (Invasive), Predicted (Noninvasive) fasting blood glucose values and its corresponding GHb% and, HbA_{1c}% values as obtained from the diabetic subjects

Diabetic Subjects	Blood Glucose Level (mg/dl)		GHb% values	HbA1c% values
	Fasting Stage			
	R-BGL (by Invasive method)	P-BGL (by Noninvasive method)		
Subject 11	141	139	9.0	6.81
Subject 12	145	136	9.1	6.89
Subject 13	181	167	10.4	7.98
Subject 14	140	144	9.0	6.81
Subject 15	148	146	9.2	6.98
Subject 16	166	154	9.9	7.56
Subject 17	159	161	9.6	7.31
Subject 18	235	143	12.3	9.58
Subject 19	165	161	9.8	7.48
Subject 20	242	142	12.6	9.83

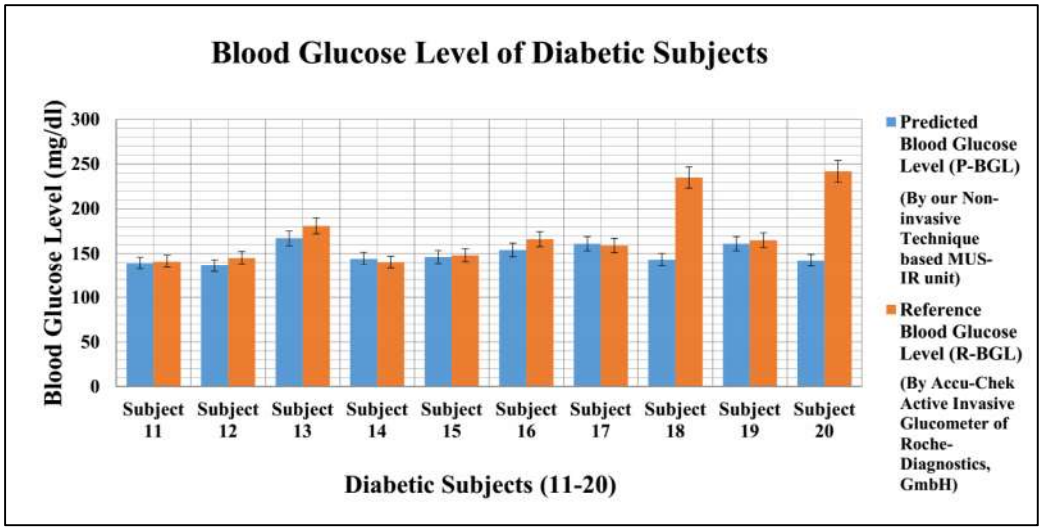


Figure 5.21: Reference (Invasive), Predicted (Noninvasive) fasting blood glucose values as obtained from the diabetic subjects; error bars indicate ±5 percentage error.

Similarly, the Table 5.13 and figure 5.21 depicts the medically significant and acceptable correlation between the invasive, noninvasive fasting blood glucose readings as obtained from the diabetic (11 to 20) study subjects during our clinical study. Again, as per conversion chart of GHb% to HbA1c% [Nathan *et al.* (1984); Bunn *et al.* (1975); Bates *et al.* (1978); Trivelli *et al.* (1971)], all the GHb% values are converted to HbA1c% values.

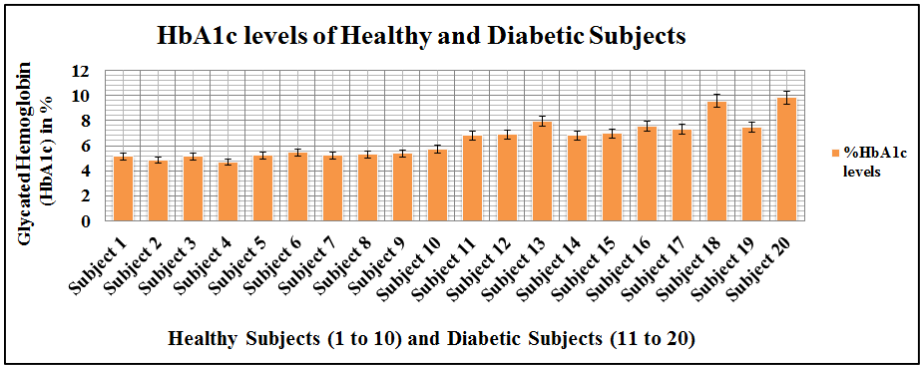


Figure 5.22: HbA1c values of the Healthy (1-10) and Diabetic (11-20) Subjects; error bars indicate ±5 percentage error.

The HbA1c values of the diabetic subjects as seen in Table 5.13 are higher as compared to the values in Table 5.12 of the normal healthy subjects. As for example, in case of subject 1 (healthy normal subject) the fasting invasive blood glucose as estimated by invasive glucometer has been 86 mg/dl. Similarly, the fasting noninvasive blood

glucose as predicted has been 99 mg/dl respectively. The GHb and HbA1c levels in percentage have been 7.0% and 5.13% respectively. Again, in case of subject 11 (Diabetic subject) the fasting invasive blood glucose as estimated by invasive glucometer has been 141 mg/dl. Similarly, the fasting noninvasive blood glucose level as predicted has been 139 mg/dl respectively. The GHb and HbA1c levels in percentage have been 9.0% and 6.81% respectively. Similarly, the figure 5.22 also reveals that diabetic subject's HbA1c values are higher as compared to normal subject's HbA1c values. This fact reveals poor glycemic control in diabetic (hyperglycemic) subjects (11-20) as compared to healthy normal subjects (1-10) respectively.

Now, the figure 5.23 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over ten healthy normal and ten diabetic study subjects respectively. The Table 5.14 depicts that 19 (95.00%) out of total 20 data pair sets, exists between the medically acceptable A and B zones respectively. Only 01(05.00%) data pair set fall on the D zone. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C and E zones respectively.

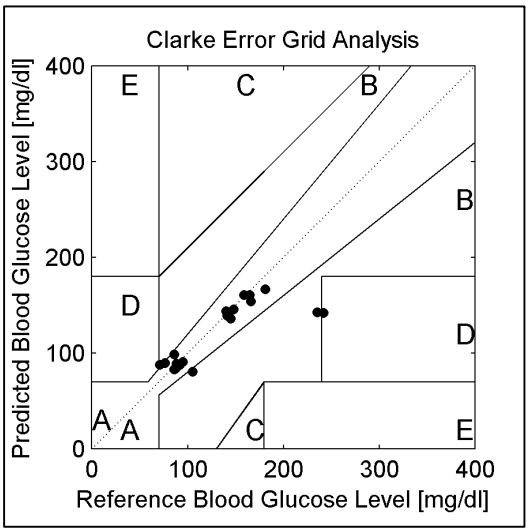


Figure 5.23: Clarke Error Grid Analysis of reference (invasive) and predicted (noninvasive) blood glucose measurements as obtained from the normal healthy subjects (1-10) and diabetic subjects (11-20) altogether respectively; error bars indicate ±5 percentage error.

Table 5.14: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	16	03	00	01	00
Percentage of total data pairs occupying A to E zones	80.00%	15.00%	00.00%	05.00%	00.00%

The Table 5.15 depicts our performance assessment values as acquired during this clinical investigation over ten healthy normal and ten diabetic study subjects. Further, the Table 5.15 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 16.35 mg/dl, 04.00 mg/dl, and 31.79 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 10.56%, and 04.27% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 19.09 mg/dl and 00.82 respectively.

Further, as depicted from Table 5.15, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

Table 5.15: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	16.35 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	04.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	31.79 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	10.56 %	(08.60 to 40.80)[d] %	
%MdARE	04.27 %	(07.70 to 30.00)[e] %	
SEP	19.09 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.82	(00.49 to 00.95)[g]	

5.6.7 Conclusion:

The Glycated hemoglobin (HbA1c) values vary largely in the diabetic subjects as compared to the healthy normal subjects. The Clarke Error Grid and the statistical analysis depict the promising aspect of our noninvasive technique based prototype (MUS-IR) unit for fasting blood glucose measurement in the human subjects.

5.7 Blood glucose and blood pressure relationship:

5.7.1 Introduction:

This section represents the clinical study over healthy normal and diabetic subjects to evaluate their respective blood glucose levels effect over blood pressure values. Large number of diabetic subject prevalence and the increasing trends of high blood pressures in them [Boer *et al.* (2008); Holman *et al.* (2008); Filipovsky *et al.* (1996)] motivated us for conducting this clinical study.

Simultaneously, a lab-based study performed to evaluate the sensitivity performance of our noninvasive technique based prototype (MUS-IR) unit in measuring blood glucose levels in healthy normal and diabetic subjects.

5.7.2 Study subjects:

In total ten (seven males, three females) adult subjects participated in this clinical study. Five subjects are healthy normal (four males, one female, age = 25.6±5.0 years, height = 155±9.0 cm, weight = 55.8±15 kg). Other, five subjects had diabetes (three males, two females, age = 28±16.5 years, height = 158±7.5 cm, weight = 60±15.8 kg). The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.7.3 Experimental protocol:

In this present work, the blood glucose (invasive and noninvasive) and blood pressure measurement performed simultaneously for three consecutive days during fasting, postprandial, and random stages of the healthy normal and diabetic subjects.

This type of clinical study evaluates the efficiency of our noninvasive technique based prototype unit in blood glucose monitoring during fasting, postprandial, and random stages along with blood pressure impact over it.

The left and right hand fingers of the study subjects serve as the measurement sites for acquisition of invasive blood samples and noninvasive signals respectively. Herein, the left arm of the study subjects serves as the blood pressure measurement site.

Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.7.4 Blood pressure and blood glucose measurement:

5.7.4.1 Blood pressure measurement:

In this present work, the digital blood pressure monitoring device of Citizen (CH-432B), Tokyo, Japan measures the blood pressure (systolic, diastolic) values of the study subjects during fasting, postprandial, and random stages for all the three consecutive days of the clinical study respectively.

This instrument measures both the systolic and diastolic blood pressure values based on korotkoff sound characterizations. The systolic blood pressure point refers to the certain point, where the korotkoff sound becomes audible. Similarly, the diastolic blood pressure point refers to the certain point where the korotkoff sound becomes inaudible or diminishes [Pickering *et al.* 2005]. The Table 5.16 depicts the blood pressure classification as per American Heart Association (2011) guidelines are as follows:

Table 5.16: Blood pressure classification in adult subjects [AHA (2011)].

Category	Systolic, mm Hg	Diastolic, mm Hg
Hypotension	< 90	< 60
Desired	90-119	60-79
Prehypertension	120-139	80-89
Stage 1: Hypertension	140-159	90-99
Stage 2: Hypertension	160-179	100-109
Hypertensive Emergency	≥ 180	≥ 110
Isolated Systolic Hypertension	≥ 140	< 90

5.7.4.2 Blood glucose measurement:

In this present work, our noninvasive technique based prototype unit measures the noninvasive blood glucose levels during fasting, postprandial, and random stages for all the three consecutive days of the clinical study.

Similarly, for cross validation, the Accu-Chek Active of Roche diagnostics GmbH, Mannheim, Germany determines the invasive blood glucose levels during fasting, postprandial, and random stages for all the three consecutive days of the clinical study.

5.7.5 Result and Discussion:

The clinical study performed during fasting, postprandial, and random stages are as follows:

5.7.5.1 Stage I:

The Table 5.17 and 5.18 depicts the fasting stage results as obtained from the healthy normal (I to V) and diabetic (VI to X) subjects during all three consecutive days of the clinical study.

As depicted from the Table 5.17, the results of the fasting healthy normal subjects (I to V) shows the regular and normal trends in blood pressure and the blood glucose levels during all the three consecutive days of the clinical investigation.

However, the Table 5.18 depicts both the fasting blood glucose levels and blood pressure values of the diabetic subjects (VI to X) are irregular and on elevated mode during all the three consecutive days of the clinical investigation.

Table 5.17: Fasting stage blood glucose and blood pressure values of the healthy normal subjects (I to V) as observed during all the three consecutive days of the clinical study.

Days	**Healthy Normal Subjects**	**Blood Glucose Level (mg/dl)**		**Blood Pressure**	
		Fasting Stage		**Systolic (mm Hg)**	**Diastolic (mm Hg)**
		R-BGL (By Invasive method)	**P-BGL (By Noninvasive method)**		
Day 1	Subject I	92	89	121	79
	Subject II	89	84	118	80
	Subject III	85	91	120	75
	Subject IV	90	85	122	85
	Subject V	88	86	120	82
Day 2	Subject I	81	88	118	78
	Subject II	83	91	112	81
	Subject III	87	84	120	76
	Subject IV	91	89	116	83
	Subject V	86	81	119	77
Day 3	Subject I	87	98	120	74
	Subject II	86	79	117	78
	Subject III	83	88	121	73
	Subject IV	89	95	124	82
	Subject V	82	92	118	84

Table 5.18: Fasting stage blood glucose and blood pressure values of the diabetic subjects (VI to X) as observed during all the three consecutive days of the clinical study.

Days	Diabetic Subjects	Blood Glucose Level (mg/dl)		Blood Pressure	
		Fasting Stage		Systolic (mm Hg)	Diastolic (mm Hg)
		R-BGL (By Invasive method)	P-BGL (By Noninvasive method)		
Day 1	Subject VI	138	130	145	100
	Subject VII	120	124	139	95
	Subject VIII	118	114	140	102
	Subject IX	139	137	139	98
	Subject X	120	117	144	101
Day 2	Subject VI	136	129	141	102
	Subject VII	131	135	136	99
	Subject VIII	126	135	134	103
	Subject IX	138	135	135	96
	Subject X	131	127	140	103
Day 3	Subject VI	133	129	137	104
	Subject VII	137	132	132	105
	Subject VIII	133	117	131	103
	Subject IX	136	126	134	93
	Subject X	128	121	137	105

5.7.5.2 Stage II:

The Table 5.19 and 5.20 depicts the postprandial stage results as obtained from the healthy normal (I to V) and diabetic (VI to X) subjects during all three consecutive days of the clinical study**.**

Table 5.19: Postprandial stage blood glucose and blood pressure values of the normal subjects (I to V) as observed during all the three consecutive days of the clinical study.

Days	Healthy Normal Subjects	Blood Glucose Level (mg/dl)		Blood Pressure	
		Postprandial Stage		Systolic (mm Hg)	Diastolic (mm Hg)
		R-BGL (By Invasive method)	P-BGL (By Noninvasive method)		
Day 1	Subject I	115	100	123	83
	Subject II	132	124	121	80
	Subject III	103	101	120	81
	Subject IV	121	127	122	82
	Subject V	109	105	119	80
Day 2	Subject I	123	116	121	79
	Subject II	134	123	115	84
	Subject III	129	133	119	79
	Subject IV	134	128	118	88
	Subject V	126	121	117	84
Day 3	Subject I	127	119	119	78
	Subject II	122	128	116	84
	Subject III	126	132	121	79
	Subject IV	131	127	113	76
	Subject V	124	134	126	85

The Table 5.19 shows that during postprandial stage both the blood glucose and blood pressure values of all the healthy normal (I to V) subjects are in the standard normal ranges during all the three consecutive days of the clinical study.

Table 5.20: The postprandial stage blood glucose and blood pressure values of the diabetic subjects (VI to X) as observed during all the three consecutive days of the clinical study.

Days	Diabetic Subjects	Blood Glucose Level (mg/dl)		Blood Pressure	
		Postprandial Stage		Systolic (mm Hg)	Diastolic (mm Hg)
		R-BGL (By Invasive method)	P-BGL (By Noninvasive method)		
Day 1	Subject VI	230	224	155	98
	Subject VII	292	280	154	102
	Subject VIII	175	170	149	99
	Subject IX	198	202	152	103
	Subject X	190	189	145	100
Day 2	Subject VI	216	188	153	99
	Subject VII	257	218	153	105
	Subject VIII	190	167	149	101
	Subject IX	225	177	154	104
	Subject X	201	153	157	102
Day 3	Subject VI	201	153	151	106
	Subject VII	222	156	152	107
	Subject VIII	205	171	144	105
	Subject IX	253	198	142	101
	Subject X	213	181	149	108

The Table 5.20 depicts that during the postprandial stages increase in blood glucose levels increases the blood pressure values in the diabetic subjects (VI to X) as observed during all the three consecutive days of the clinical study.

5.7.5.3 Stage III:

The Table 5.21 and 5.22 depicts the random stage results as obtained from the healthy normal (I to V) and diabetic (VI to X) subjects during all the three consecutive days of the clinical study.

Table 5.21: Random stage blood glucose and blood pressure values of the healthy normal Subjects (I to V) as observed during all the three consecutive days of the clinical study.

Days	Healthy Normal Subjects	Blood Glucose Level (mg/dl)		Blood Pressure	
		Random Stage		Systolic (mm Hg)	Diastolic (mm Hg)
		R-BGL (By Invasive method)	P-BGL (By Noninvasive method)		
Day 1	Subject I	80	86	120	80
	Subject II	95	91	119	82
	Subject III	88	86	123	79
	Subject IV	98	94	121	81
	Subject V	89	85	118	83
Day 2	Subject I	93	87	117	78
	Subject II	87	77	122	80
	Subject III	81	93	116	83
	Subject IV	95	116	113	88
	Subject V	99	87	119	82
Day 3	Subject I	87	81	118	79
	Subject II	91	84	115	81
	Subject III	85	92	126	71
	Subject IV	97	85	117	84
	Subject V	94	112	113	85

The Table 5.21 shows that both the Random blood glucose and blood pressure values of the healthy normal (I to V) subjects are in the standard normal ranges during all the three consecutive days of the clinical study.

Table 5.22: Random stage blood glucose and blood pressure values of the diabetic subjects (VI to X) as observed during all the three consecutive days of the clinical study.

Days	Diabetic Subjects	Blood Glucose Level (mg/dl)		Blood Pressure	
		Random Stage		Systolic (mm Hg)	Diastolic (mm Hg)
		R-BGL (By Invasive method)	P-BGL (By Noninvasive method)		
Day 1	Subject VI	138	140	142	101
	Subject VII	118	111	145	102
	Subject VIII	135	131	141	99
	Subject IX	165	158	139	92
	Subject X	130	136	147	102
Day 2	Subject VI	172	147	141	99
	Subject VII	188	133	139	95
	Subject VIII	167	148	140	100
	Subject IX	183	174	146	101
	Subject X	190	169	138	97
Day 3	Subject VI	154	142	146	98
	Subject VII	153	132	142	105
	Subject VIII	152	139	137	99
	Subject IX	176	164	141	100
	Subject X	159	149	146	102

The Table 5.22 depicts that during the random stages increase in blood glucose levels increases the blood pressure values in the diabetic subjects (VI to X) as observed during all the three consecutive days of the clinical study.

The figures 5.24 and 5.25 depicts one of the sample figures from the healthy (healthy normal subject II) and diabetic (diabetic subject IX) subjects as observed during Day 1 respectively. The error bars in figures 5.24 and 5.25 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and

predicted (noninvasive) blood glucose level data pairs are considered here. The figure 5.24 depicts that during fasting, postprandial, and random stage of healthy normal Subject II, both the invasive and noninvasive blood glucose levels are 89 mg/dl and 84 mg/dl, 132 mg/dl and 124 mg/dl, 95 mg/dl and 91 mg/dl respectively. Similarly, during those corresponding stages, the blood pressures as measured are 118/80 mm Hg (during fasting stage); 121/80 mm Hg (during postprandial stage); and 119/82 mm Hg (during random stage) respectively.

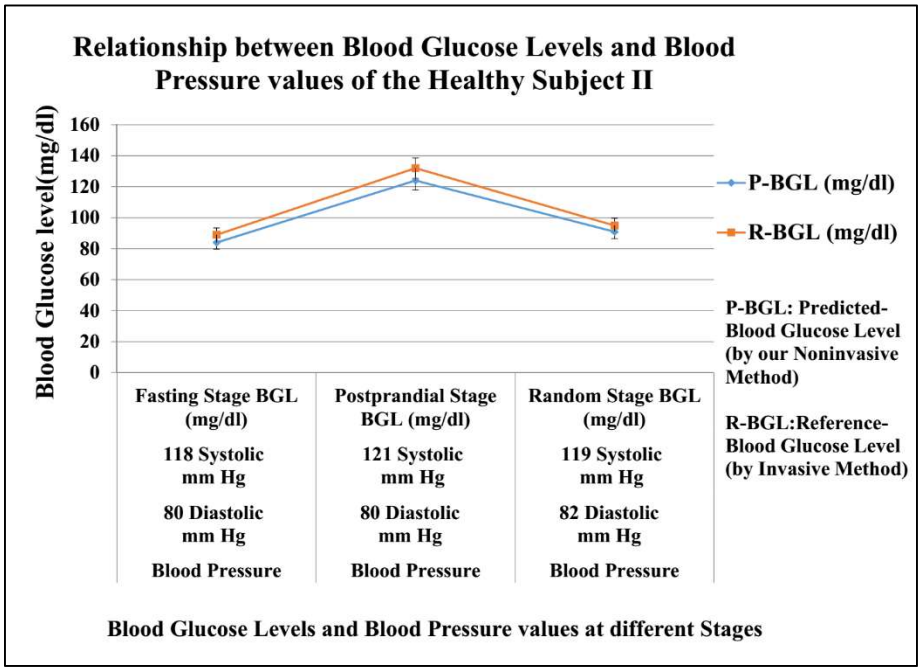


Figure 5.24: The relationship between blood glucose and blood pressure values of healthy normal subject II as observed during Day 1 of the clinical study; error bars indicate ±5 percentage error.

Now, the figure 5.25 depicts that during Fasting, Postprandial, and Random Stage of the diabetic subject IX, both the invasive and noninvasive blood glucose levels are 139 mg/dl and 137 mg/dl, 198 mg/dl and 202 mg/dl, 165 mg/dl and 158 mg/dl respectively. Similarly, during those corresponding stages, the blood pressures as measured are 139/98 mm Hg (during fasting stage); 152/103 mm Hg (during postprandial stage); and 146/101 mm Hg (during random stage) respectively.

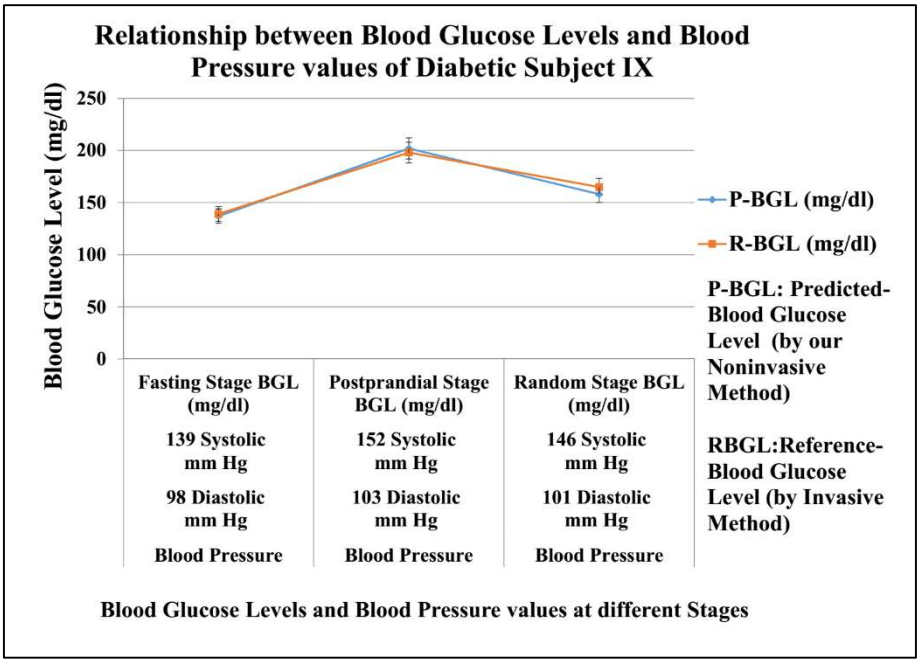


Figure 5.25: The relationship between Blood Glucose Levels and Blood Pressure values of Diabetic Subject IX respectively; error bars indicate ±5 percentage error.

Hence, the Tables 4.17, 4.19 and 4.21 depicts the normal trend of blood glucose and blood pressure values in case of all the healthy normal (I to V) subjects during all the three consecutive days of the clinical study. On the contrary, the Tables 4.18, 4.20 and 4.22 represents that blood pressure increases with the increase in blood glucose levels in case of all the diabetic (VI to X) subjects during all the three consecutive days of the clinical study.

This phenomenon indicates controlling blood glucose levels will reduce progression of long-term blood pressure related medical complications in diabetic subjects [Danaei *et al.* (2011); Wild *et al.* (2003)].

The present clinical study proves that blood glucose levels and blood pressure are interlinked and associated with each other in many pathological consequences of the diabetic subjects. Now, to evaluate the performance of our indigenously developed noninvasive technique based prototype (MUS-IR) unit, we have evaluated both the reference (invasive) and predicted (noninvasive) blood glucose readings by the Clarke Error Grid and statistical analysis respectively.

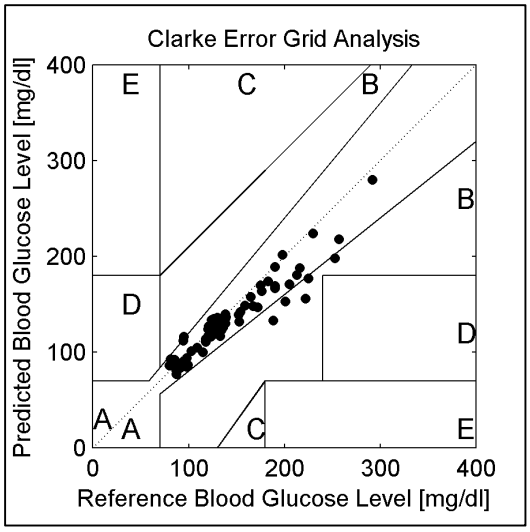


Figure 5.26: The Clarke Error Grid Analysis of the blood glucose levels as obtained from Healthy Normal (I to V) and Diabetic (VI to X) Subjects respectively.

Now, the figure 5.26 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over five healthy normal and five diabetic study subjects respectively. In Table 5.23, the Clarke Error Grid analysis shows the percentage of the total data pairs (90) falling in the zones A, B, C, D, and E are 92.22% (83 data pairs), 07.78% (07 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 90 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.23: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	83	07	00	00	00
Percentage of total data pairs occupying A to E zones	92.22%	07.78%	00.00%	00.00%	00.00%

Table 5.24: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	12.04 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	07.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	17.96 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	07.78 %	(08.60 to 40.80)[d] %	
%MdARE	05.57 %	(07.70 to 30.00)[e] %	
SEP	11.61 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.94	(00.49 to 00.95)[g]	

The Table 5.24 depicts our performance assessment values as acquired during this clinical investigation over five healthy normal and five diabetic study subjects. Further,

the Table 5.24 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 12.04 mg/dl, 07.00 mg/dl, and 17.96 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 07.78%, and 05.57% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 11.61 mg/dl and 00.94 respectively.

Further, as depicted from Table 5.24, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.7.6 Conclusion:

The clinical study based experimental result emphasizes that high blood pressure exists during elevated blood glucose levels in the diabetic subjects. This fact must play a significant role in the treatment plan for proper diabetic condition management.

Further, as per Clarke Error Grid and statistical analysis our indigenously developed noninvasive technique has been sensitive and efficient in determining blood glucose levels of healthy normal and diabetic subjects respectively.

5.8 Extended clinical study using OGTT and Random Blood Glucose Level Tests:

5.8.1 Introduction;

In this present work, the oral glucose tolerance test and random blood glucose test based clinical study performed over human subjects to measure the performance of our noninvasive technique based prototype unit. Further, the Clarke Error Grid and Statistical analysis applied here, to measure the efficiency of our noninvasive technique based prototype unit in monitoring of blood glucose levels.

5.8.2 Study subjects:

In total sixty adult subjects participated in this clinical study. Out of which, thirty healthy adult subjects participated for OGTT analysis. For performing thirty subjects OGTT, we conducted the test over six subjects per day for five consecutive days. Next, thirty adult subjects participated in one day session for Random blood glucose level tests. In which, eighteen subjects are healthy normal, seven subjects had pre-diabetes, and five subjects had diabetes. All the pre-diabetic and diabetic subjects followed their normal routine of meal intake and medications. The mean ± standard deviations of age is 40±4 years and mean ± standard deviations of body mass index is 26.2±2 kg/m^2. Overall, forty-four subjects were male and sixteen subjects were female. The clinical study reported here are in accordance with the standard ethical procedures and performed with the informed consent of all the respective study subjects. The Ethical committee of IMS-BHU, Varanasi approved the clinical study.

5.8.3 Experimental Procedures:

In this present work, the clinical study consists of two phases (oral glucose tolerance test and random test) to validate the clinical correlation between the invasive technique and noninvasive technique based blood glucose levels. During the first phase, we have performed oral glucose tolerance tests over healthy subjects after their overnight fasting. The duration of each experiment was 2 hour and 45 minutes (10 to 15 minutes for baseline observation before intake of 75 g glucose solution). The invasive and noninvasive data recorded every 30 minutes from the left and right-hand fingers respectively. In this present work, our noninvasive technique based prototype unit performs the predicted (noninvasive) blood glucose measurements. Similarly, for cross validation, the Accu-Chek Active of Roche Diagnostics GmbH, Mannheim, Germany measures the reference (invasive) blood glucose levels in the human subjects. Throughout the investigation, the study subjects remain static to reduce motion artifacts and intake of any food or liquids were restricted. During the second phase, we have performed Random Blood glucose level analysis (both invasive and noninvasive) over normal, pre-diabetic, and diabetic subjects respectively. Further, the Clarke Error Grid and statistical analysis applied here to measure the performance metrics of our noninvasive technique based prototype unit in measuring blood glucose levels of human subjects.

5.8.4 Result and Discussion:

5.8.4.1 Oral glucose tolerance test based result analysis:

The figures from 5.27 to 5.32 depict the sequential variations in the reference (invasive) and predicted (noninvasive) blood glucose concentration of all the 30 human subjects (five subjects per day) as obtained during six-day OGTT sessions.

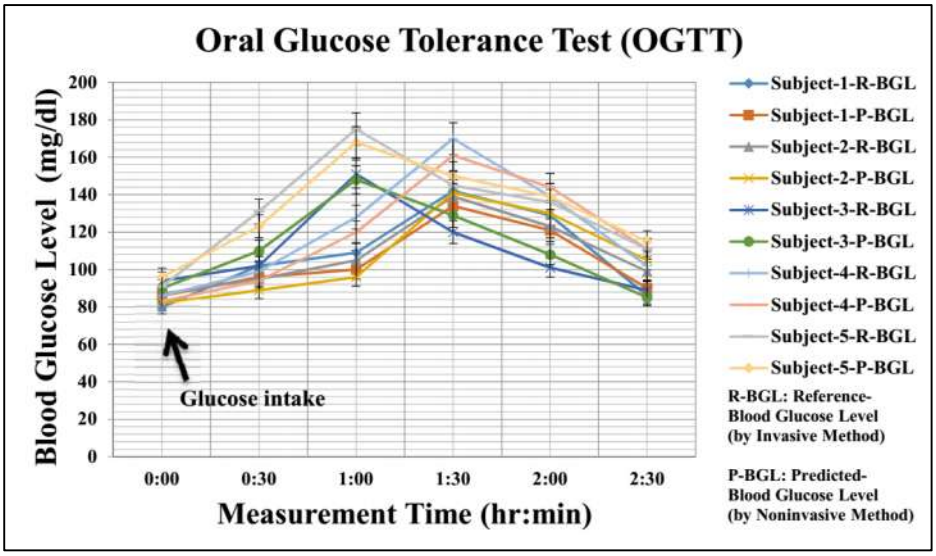


Figure 5.27: OGTT response curve of the study subjects (1 to 5) on 1st day; error bars indicate ±5 percentage error.

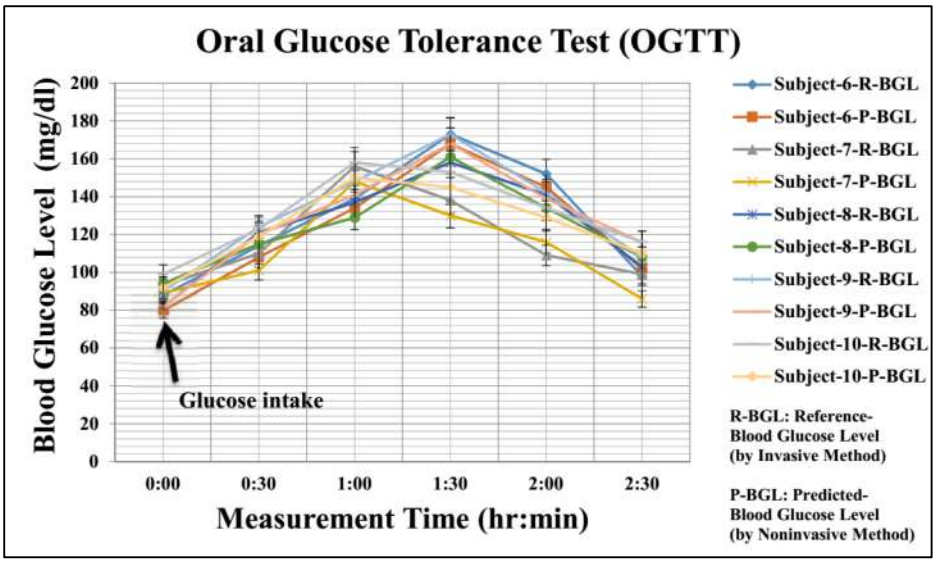


Figure 5.28: OGTT response curve of the study subjects (6 to 10) on 2nd day; error bars indicate ±5 percentage error.

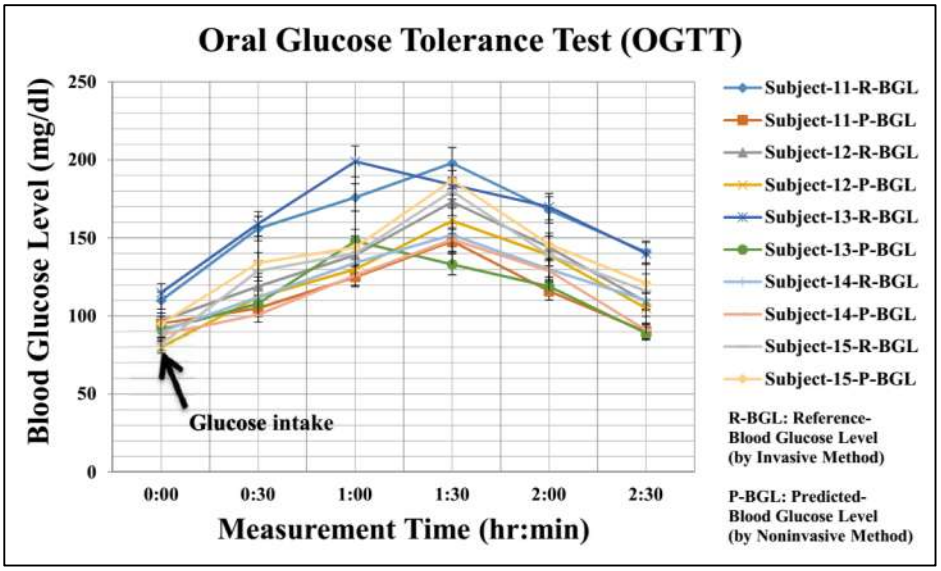


Figure 5.29: OGTT response curve of the study subjects (11 to 15) on 3rd day; error bars indicate ±5 percentage error.

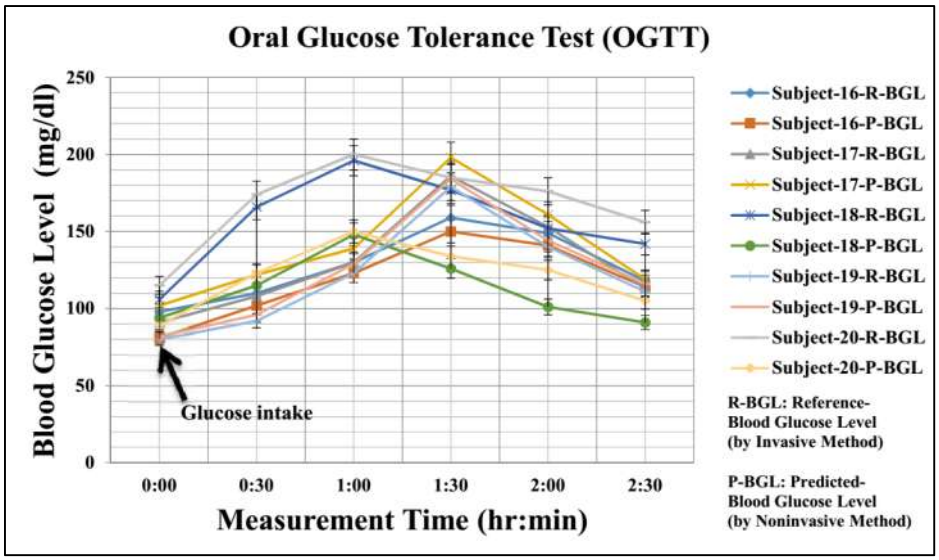


Figure 5.30: OGTT response curve of the study subjects (16 to 20) on 4th day; error bars indicate ±5 percentage error.

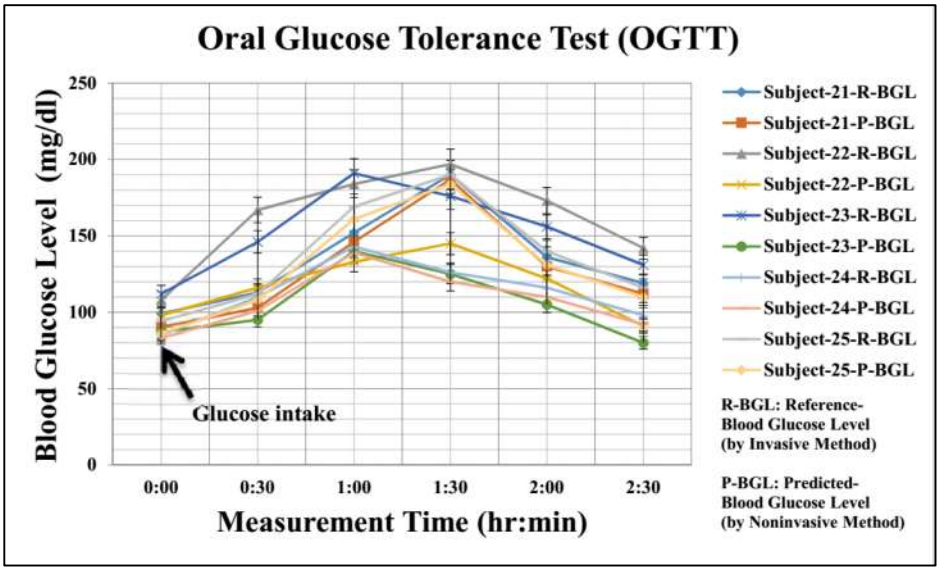


Figure 5.31: OGTT response curve of the study subjects (21 to 25) on 5th day; error bars indicate ±5 percentage error.

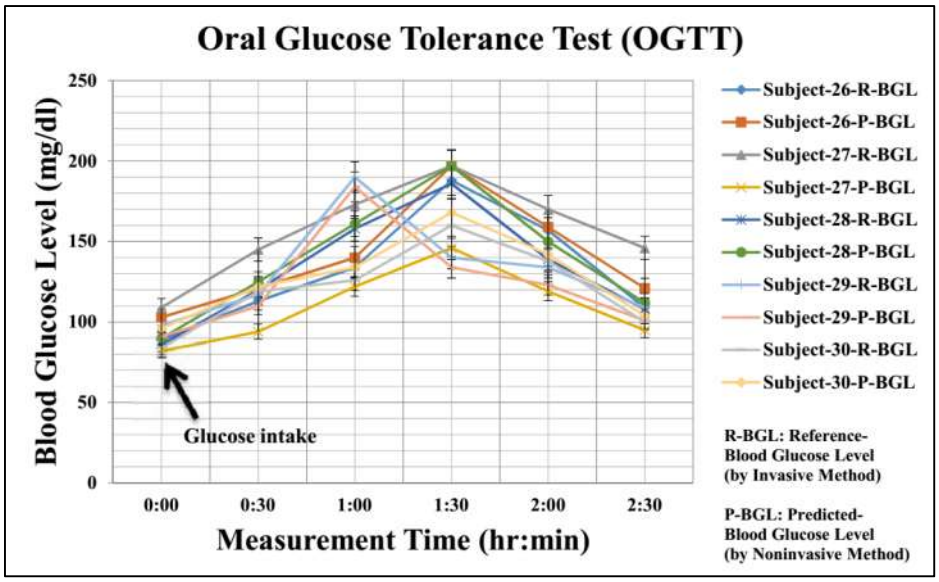


Figure 5.32: OGTT response curve of the study subjects (26 to 30) on 6th day; error bars indicate ±5 percentage error.

The error bars in figures 5.27 to 5.32 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

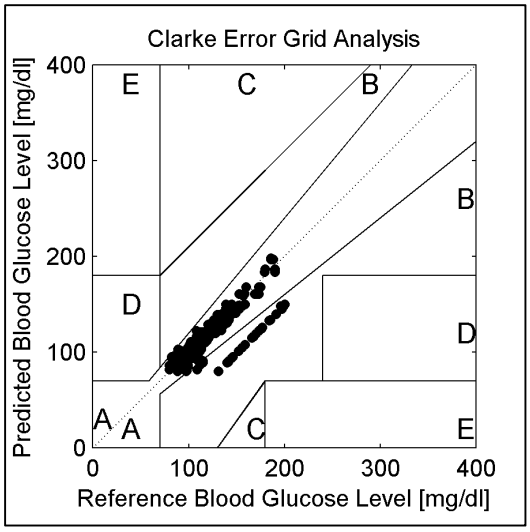


Figure 5.33: Clarke Error Grid analysis based plot for reference (invasive) and predicted (noninvasive) blood glucose measurement as obtained from 30 human subjects during OGTT analysis.

Now, the figure 5.33 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during this clinical investigation over 30 healthy normal study subjects respectively. In Table 5.25, the Clarke Error Grid analysis shows the percentage of the total data pairs (180) falling in the zones A, B, C, D, and E are 78.33% (141 data pairs), 21.67% (39 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 180 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

Table 5.25: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	141	39	00	00	00
Percentage of total data pairs occupying A to E zones	78.33%	21.67%	00.00%	00.00%	00.00%

Table 5.26: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	**Our assessment based values**	**Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data**	
		Published data ranges	**References**
MAE	15.92 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin (2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	08.00 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	23.76 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	11.09 %	(08.60 to 40.80)[d] %	
%MdARE	05.97 %	(07.70 to 30.00)[e] %	
SEP	17.67 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.76	(00.49 to 00.95)[g]	

The Table 5.26 depicts our performance assessment values as acquired during OGTT based clinical investigation over thirty healthy normal study subjects. Further, the

Table 5.26 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 15.92 mg/dl, 08.00 mg/dl, and 23.76 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 11.09%, and 05.97% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 17.67 mg/dl and 00.76 respectively.

Further, as depicted from Table 5.26, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.8.4.2 Random blood glucose test based analysis:

The figures from 5.34 to 5.36 represents the 30 data pairs of reference (invasive) and predicted (noninvasive) blood glucose concentration as obtained from the 30 human subjects during one-day session of Random blood glucose measurement.

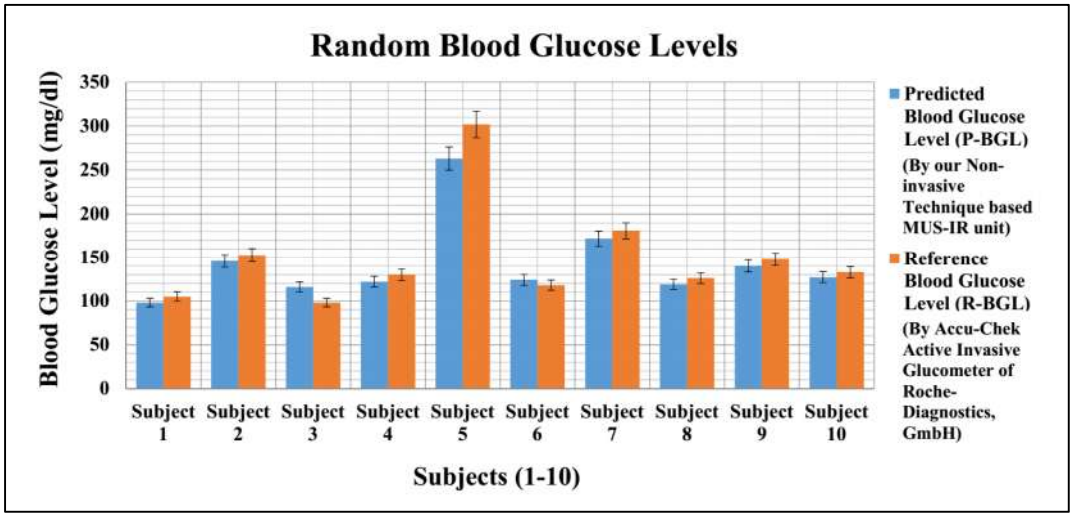


Figure 5.34: Random Blood Glucose tests of the Subjects (1-10); error bars indicate ±5 percentage error.

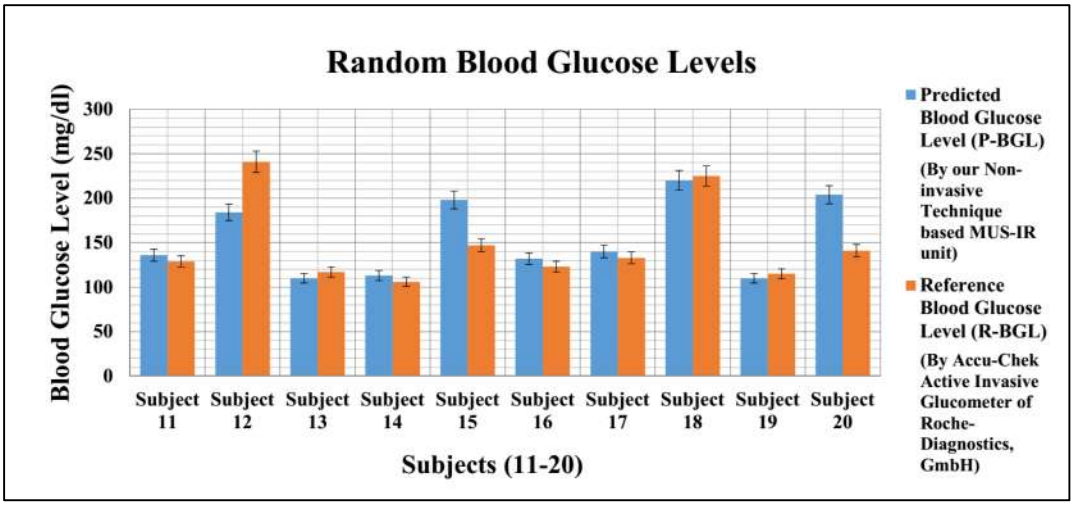


Figure 5.35: Random Blood Glucose tests of the Subjects (11-20); error bars indicate ±5 percentage error.

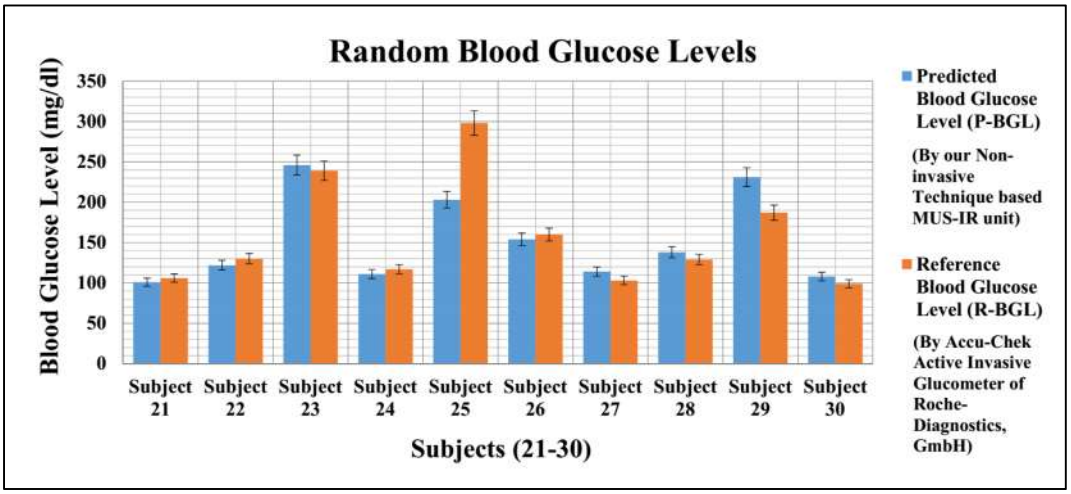


Figure 5.36: Random Blood Glucose tests of the Subjects (21-30); error bars indicate ±5 percentage error.

The error bars in figures 5.34 to 5.36 shows the ±5% error values and the solid lines correspond to the data calculated using both the methods respectively. Hence, for further calculations the mean values of the respective reference (invasive) and predicted (noninvasive) blood glucose level data pairs are considered here.

Now, the figure 5.37 depicts Clarke Error Grid Analysis of all the reference (invasive) and predicted (noninvasive) blood glucose data pair sets as obtained during Random blood glucose test based clinical investigation over 30 study subjects

respectively. In Table 5.27, the Clarke Error Grid analysis shows the percentage of the total data pairs (30) falling in the zones A, B, C, D, and E are 83.33% (25 data pairs), 16.67% (05 data pairs), 00.00% (00 data pairs), 00.00% (00 data pairs), and 00.00% (00 data pairs), respectively. Hence, all the 30 data pairs occupy the medically significant A and B zones respectively. Further, none of the data pair sets occupies medically insignificant and potentially dangerous C to E zones respectively.

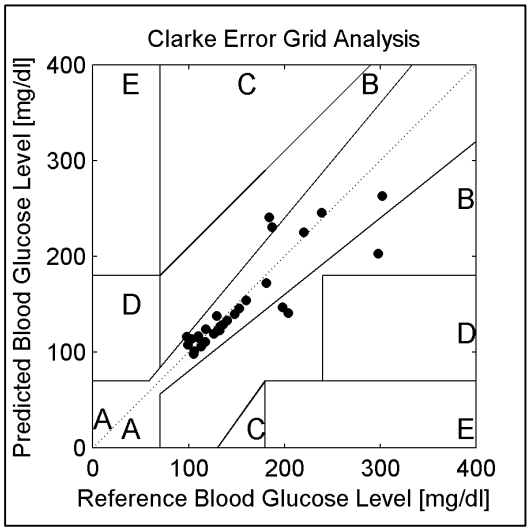


Figure 5.37: Clarke Error Grid analysis based plot for reference (invasive) and predicted (noninvasive) blood glucose measurement as obtained from 30 human subjects during Random blood glucose tests.

Table 5.27: Clarke Error Grid Analysis of Reference (Invasive) and Predicted (Noninvasive) Blood Glucose Levels

Clarke Error Grid Analysis					
Zones	**A Zone**	**B Zone**	**C Zone**	**D Zone**	**E Zone**
	Medically accurate	**Medically acceptable**	**Medically insignificant and potentially harmful**		
Total number of data pairs occupying A to E zones	25	05	00	00	00
Percentage of total data pairs occupying A to E zones	83.33%	16.67%	00.00%	00.00%	00.00%

Table 5.28: Performance summary and comparison with other noninvasive techniques and CGMS(s) based published data.

Statistic name	Our assessment based values	Comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS)s based published data	
		Published data ranges	References
MAE	17.76 mg/dl	(07.00 to 30.00)[a] mg/dl	Amir *et al.* (2007)[d]; Boeckle *et al.* (2002)[a,d,e]; Boehm *et al.* (2010)[a,d,e]; Boehm *et al.* (2009)[a,d,e]; Caduff *et al.*(2011)[d]; Caduff *et al.*(2009)[d]; Enejder *et al.*(2005)[a]; Guevara *et al.* (2010)[c]; Gabbay *et al.*(2008)[d,e]; Heise *et al.*(2009)[a,f]; Lipson *et al.* (2009)[d,e]; Malchoff *et al.* (2002)[d]; Mohammadi *et al.*(2014)[d]; Myllyla *et al.* (2009)[a]; Pai *et al.* (2015)[d]; Ozaki *et al.* (2009)[c,f]; Oliver *et al.* (2008)[g]; Ramchandani *et al.*(2012)[d]; Robinson *et al.* (1992)[a]; Tuchin.(2009)[a,c,f,g]; Tamada *et al.* (1999)[d]; Vashist (2013)[d]; Valgimigli *et al.* (2010)[a,b,e]; Vaddiraju *et al.*(2010)[g]; Weiss *et al.*(2007)[d,e]; Yoon *et al.* (2009)[f]; Yadav *et al.* (2015)[f]; Zhao *et al.* (2002)[a,e]; Zilberman *et al.* (2009)[e].
MdAE	07.50 mg/dl	(10.40 to 19.10)[b] mg/dl	
RMSE	28.20 mg/dl	(25.00 to 46.00)[c] mg/dl	
%MARE	10.10 %	(08.60 to 40.80)[d] %	
%MdARE	06.15 %	(07.70 to 30.00)[e] %	
SEP	24.88 mg/dl	(10.00 to 54.00)[f] mg/dl	
r value	00.85	(00.49 to 00.95)[g]	

The Table 5.28 depicts our performance assessment values as acquired during Random BGL test based clinical investigation over 30 study subjects. Further, the Table

5.28 shows the results comparison with other Noninvasive Techniques and Continuous Glucose Monitoring System(s) (CGMS) based published data ranges. The performance metrics based errors such as MAE (Mean Absolute Error), MdAE (Median Absolute Error), and RMSE (Root Mean Squared Error) values were 17.76 mg/dl, 07.50 mg/dl, and 28.20 mg/dl respectively. Similarly, the performance metrics based percentage errors such as Percentage-MARE (Percentage of Mean Absolute Relative Error), and Percentage-MdARE (Percentage of Median Absolute Relative Error) values were 10.10%, and 06.15% respectively. The SEP (Standard Error of Prediction) and Pearson's Correlation Coefficient (r) values were 24.88 mg/dl and 00.85 respectively. Further, as depicted from Table 5.28, the output results obtained by our noninvasive technique are better than or comparable with other noninvasive blood glucose monitoring techniques. Further, its accuracy levels are also akin with other commercially existing Continuous Glucose Monitoring Systems. Hence, all these overlaid accuracy measures based statistical analysis depicts the strong promising aspect of our noninvasive technique for blood glucose measurement in the human subjects.

5.8.5 Conclusion:

We have represented the indigenously designed modulated ultrasound and Infrared technique based technique for noninvasive blood glucose measurement on human subjects. The in-vivo results showed good correlation in blood glucose measurement. The result of the Clarke error grid analysis, statistical evaluations values validates that our noninvasive system is potentially capable in performing noninvasive blood glucose measurement over human subjects. Our noninvasive system was medically safe, easy, and acceptable, as reflected by the overall study subject's well-tolerated compliances. Therefore, a new technique for noninvasive blood glucose measurement using modulated ultrasound and infrared technique is developed and the observation validates the supposition of the new concept. All this clinical correlation based clinical study results direct towards the reliable efficiency of the indigenously developed amplitude modulated ultrasound and infrared technique based noninvasive system for blood measurement in human subjects. Further, in next chapter our investigation includes comparison of our overall blood glucose measurement data with the published data in literatures and statistical analysis to evaluate the statistical significance of our results.