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Correlation of Serum Adenosine Deaminase (ADA) with Glycated Heamoglobin (HbAcl) in Type 2 Diabetics Mellitus Patients: A Single Center Study in Bangladesh

Mita Bhowmik[1*], Pulak Kumar Dey[2], Qazi Shamima Akhter[3]

[1]Assistant Professor, Department Physiology, Dr. Sirajul Islam Medical College & Hospital, Dhaka, Bangladesh.
Email: mitabhowmik34@gmail.com,
Orcid ID: 0000-0001-8597-8865
[2]Assistant Professor, National Institute of Diseases of the Chest (NIDCH), Dhaka, Bangladesh.
Email: pkdey_25@yahoo.com,
Orcid ID: 0000-0002-8233-8018
[3]Professor & Head, Department of Physiology, Dhaka Medical College & Hospital, Dhaka, Bangladesh.
Email: qazishamimaakhter@yahoo.com,
Orcid ID: 0000-0002-7589-899X

*Corresponding author

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Abstract

Background: Worldwide, diabetes mellitus is a major health problem leading to remarkable mortality and morbidity day by day. Adenosine deaminase (ADA) catalyzes the irreversible deamination of adenosine to inosine is a polymorphic enzyme which is considered to be related to type 2 diabetes mellitus (T2DM). So, it may be useful in formulating proper guideline for evaluating the glycemic status in T2DM. The aim of the study was to assess the correlation of serum adenosine deaminase (ADA) with glycated heamoglobin (HbA1c) in type 2 diabetics mellitus patients. **Material & Methods:** It was a cross sectional study and conducted in the Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh from July 2014 to June 2015. A total number of 150 participants of both sexes were included in this study as the study population. The samples were divided into major two groups. The control group (Group A) consisted of 50 age matched healthy subjects for comparison and the study group (Group B) consisted of 100 type 2 diabetic patients with the age ranging from 40-55 years. Again, depending on HbA1c level, the study group was divided into two subgroups. Group B1 consisted of 50 participants with HbA1c<6.5% and Group B2 consisted of 50 participants with HbA1c>6.5%. The study parameters were including serum ADA, FPG, HbA1c. For statistical analyses one-way ANOVA test, unpaired Student's test and Pearson's correlation coefficient (r) test were performed as applicable using SPSS version 20.0. **Results:** type-2 DM as compared to healthy controls. Again, serum ADA (P<0.001) levels were significantly higher in type2DM with HbA1c ≥6.5% than that of type2 DM with HbA1c <6.5%. There is a positive correlation of serum ADA with FPG, HbA1c in type 2 diabetic patients. **Conclusion:** After analyzing the results of the study, it is concluded that estimation of serum ADA level might be used as a new marker for prediction of glycemic in type2 diabetes mellitus.

Keywords:- Correlation, Serum Adenosine Deaminase, Glycated Heamoglobin, HbAcl, Type 2 diabetics mellitus.

INTRODUCTION

Diabetes mellitus is a major global health problem. It is a metabolic disorder characterized by chronic hyperglycemia with derangement of carbohydrate, fat and protein metabolism due to absolute or relative deficiency of insulin secretion and action or both.[1] By the year 2030, about 29.6 million people will be diabetic in USA, 101.2 million in India and 16.8 million in Bangladesh.[2] Prevalence of diabetes in rural population in Bangladesh was found to be about 7.2% in the

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year of 2011.[3] For the diabetic patients, insulin resistance is a complex phenomenon where several genetic defects combine with environmental stresses, such as obesity or infections.[4] Insulin sensitivity is influenced by a number of factors including age, weight, ethnicity, body fat (especially abdominal), physical activity, dietary habits (excessive carbohydrate diet), free fatty acid, vitamin D deficiency, inflammation, genetic factors, medications such as glucocorticoid and adenosine molecule. Hyperglycemia and hyperinsulinemia both of them can impair insulin secretion and insulin sensitivity. According to Verma et al.[5] HbA1c and insulin levels significantly increase with the duration of diabetes and showed a significant correlation for age, sex and duration of diabetes. Adenosine deaminase, an enzyme of purine metabolism which is present in red cells and vessel wall. It has two isoenzyme forms: ADA1 and ADA2 which are coded by two separate genes. It acts on adenosine and other adenosine nucleoside analogues and catalyzes the irreversible hydrolytic deamination of adenosine to inosine and 2′-deoxyadenosine to 2′-deoxyinosine. Both inosine and 2′-deoxyinosine are converted to hypoxanthine, xanthine and finally to uric acid.[6,7] Adenosine deaminase is a cytosolic enzyme, which has been the subject of considerable interest in recent years. Literature suggests that the levels of adenosine are reduced by ADA. Adenosine mimics the action of insulin on glucose and lipid metabolism in adipose tissue and skeletal muscle and responsible for increasing glucose uptake into cells.[8] It has also an antilipolytic property by which it reduces free fatty acid level. High ADA level tends to decrease. glucose uptake into cells and increase free fatty acid level.[9,10] Many Studies have shown that the level of ADA is higher in patients with type-2 DM than that in non-diabetics.[11,12] Adenosine deaminase distributed in human tissues was considered as good marker of cell mediated immunity.[13] Chronic hyperglycemia leads to increased oxidative stress by forming enediol radicals and superoxide ions by NADPH oxidase system and increases ADA levels, both leading to insulin resistance. GLUT4 receptors are downregulated in the absence of adenosine.[14] Gowda et al.[15] performed a cross sectional study on type2 diabetic patient to evaluate the role of serum ADA as a marker. They found that serum ADA activity was increased in the diabetic patients. Several studies suggested that, increased serum ADA level in type2 DM and its positive correlation with HbA1c.[16,17] On the contrary; Shantaram, Anusha and Chethana conducted a study on 20 normal healthy individuals and 20 patients with type 2DM.[18] Serum ADA level was estimated in all subjects under study. They observed that serum ADA levels were significantly decreased in the patients of type 2 DM and also reported the association of low serum ADA level in type2 DM.[19] From the above studies, it has been revealed that increased serum ADA level in patients with type2 DM may be associated with HbA1c. So, the present study is intended to assess the serum ADA level and its relationship with glycated hemoglobin (HbA1c), in Bangladeshi patients with type2 DM.

Objectives

General objective

To assess the relationship of serum adenosine deaminase (ADA) with glycated heamoglobin (HbA1c) in T2DM patients.

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Specific objectives

- To estimate glycated hemoglobin (HbA1c) level in T2DM patients to assess their glycemic status.
- To estimate serum adenosine deaminase (ADA) level in T2DM patients to assess their enzyme status.

MATERIAL AND METHODS

It was a cross sectional study followed purposive sampling method. It was conducted at the Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh during the period from July 2014 to June 2015. A total number of 150 participants of both sexes were included in this study as the study population. The samples were divided into major two groups. The control group (group A) consisted of 50 age matched healthy participants for comparison and the study group (group B) consisted of 100 type 2 diabetic patients with the age ranging from 40-55 years. Again, depending on HbA1c level, the study group was divided into two subgroups. Group B1 consisted of 50 subjects with HbA1c<6.5% and group B2 consisted of 50 participants with HbA1c>6.5%. The study subjects (group B) were selected from outpatient Department of Dhaka Medical College Hospital and BIRDEM General Hospital, Dhaka, Bangladesh. The control (group A) were selected by personal contact from different areas of Dhaka city. Group B1 patient's diagnosis was done by HbA1c (<6.5%). And group B2 patients were also diagnosed HbA1c (> 6.5%). Informed written consent was taken from the participants. Detailed family and medical history were taken before taking blood. Anthropometric measurement of the participants was done and blood pressure was measured. With aseptic precaution, 10 ml of venous blood was collected from ante-cubital vein by a disposable plastic syringe from each subject for estimation of biochemical tests. HbA1c, serum ADA, were estimated in the laboratory of the Department of Biochemistry, Department of Microbiology & Immunology of BSMMU and Department of Laboratory Services of National Institute of ENT, Dhaka. With all aseptic precautions 10 ml blood from each study population was collected from ante-cubital vein by disposable plastic syringe. From that sample 3 ml whole blood was taken in an EDTA containing tube for estimation of HbA1c. About 2.2 ml blood was taken (1:9 ratio) in one test tube containing 3.8% trisodium citrate. Then the blood sample was centrifuged at 1500rpm for 15 minutes at room temperature. The remaining blood was transferred in a de-ionized glass test tube and kept in slanted position till formation of clot. Then blood sample was centrifuged at a rate of 3000 rpm for 5 minutes. After that, supernatant serum was collected in labeled Eppendorf tube and preserved in a refrigerator until analytical measurement of serum ADA level were done. Percentage of HbA1c was measured in whole blood by a variant hemoglobin testing system (Bio-Rad model) using a modified HPLC method. All the parameters were expressed as mean ± SD and range. Comparison among the groups was done by one-way ANOVA test and unpaired Student's test. Pearson's correlation coefficient (r) test was performed to explore the association between study parameters. Statistical analyses were performed by using a computer based statistical program SPSS version 20.0.

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RESULTS

In this study, the mean (± SD) age in groups A, B1 and B2 were 45.00±5.17, 45.96±5.26 and 45.94±5.29 years respectively. No statistical differences were observed among these three groups. Therefore, all the groups were matched for age. The mean (±SD) BMI in groups A, B1 and B2 were 22.01±1.66, 23.35±2.29 and 25.35±1.77 kg/m2 respectively and the mean (±SD) systolic blood pressure in groups A, B1 and B2 were 120.80±7.98, 130.40±8.32and 136.50±9.60 mm of Hg respectively. The mean (±SD) diastolic blood pressure in groups A, B1 and B2 were 77.40±5.17, 82.60±4.43and 85.90±4.25 mm Hg respectively. The differences of the mean value among the three groups were statistically significant (p<0.001). Again, the mean fasting plasma glucose was significantly (p<0.001) higher in group B2 than that of group B1. The mean (±SD) HbA1c level in groups A, B1 and B2 was 4.70±0.46, 6.08±0.33 and 9.71±2.41 respectively. The differences of the mean value among the three groups were statistically significant (p<0.001). In this study, the mean HbA1c level was significantly higher in group B1 (P<0.001) and B2 (P<0.001) as compared to group A. Again, the mean HbA1c was significantly (p<0.001) higher in group B2 than that of group B1. The mean (±SD) serum ADA level was 10.48±2.22, 17.75±2.25and 30.58±6.73 U/L in groups A, B1 and B2 respectively. The differences of the mean value among the three groups were statistically significant (p<0.001). In this study, the mean (±SD) ADA level was significantly higher in group B1 (p<0.001) and B2 (p<0.001) in comparison to that of group A. Again, the mean (±SD) ADA level was significantly (p<0.001) higher in group B2 than that of group B1. The differences of the mean value among the three groups were statistically significant (p<0.001). In this study, in group B1 (n=50), ADA level ≤15.0 was found in 7(14.0%) participantsand > 15.0 U/L was found in 43(86.0%) participants. Again, in group B2 (n=50), ADA level ≤15.0 U/L was found in 0% participants and > 15 U/L was found in 50(100%) participants. In group B1, serum ADA level showed positive correlation (r=+0.346) which was statistically significant. Again, in group B2, serum ADA level showed positive correlation (r=+0.303). which was statistically significant association. In group B1, serum ADA level showed positive correlation (r=+0.460) with HbA1c, which was statistically significant. Again, in group B2, serum ADA level showed positive correlation (r=+0.452) with HbA1c, which was statistically significant association. In group B1, serum ADA level showed positive correlation (r=+0.344). which was statistically significant. Again, in group B2, serum ADA level showed positive correlation (r=+0.302), which was statistically significant association.

Table 1: General characteristics of the participants in different groups (N=150)

Parameters		**Group-A (n=50)**	**Group-B1 (n=50)**	**Group-B2 (n=50)**
		Healthy People	**(HbA1c <6.5%)**	**(HbA1c≥6.5%)**
		n(%)/ Mean ±SD	**n(%)/ Mean ±SD**	**n(%)/ Mean ±SD**
Age (Years)		45.00±5.17	45.96±5.26	45.94±5.29
Gender	Male	23(46.0)	24(48.0)	27(54.0)
	Female	27(54.0)	26(52.0)	23(46.0)

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BMI (kg/m2)	Value	22.01±1.66	23.35±2.29	25.35±1.77
	Range	(19.0-25.0)	(19.0-29.30)	(22.80-29.30)
SBP	Value	120.80±7.98	130.40±8.32	136.50±9.60
	Range	(100.0-140.0)	(110.0-150.0)	(120.0-150.0)
DBP	Value	77.40±5.17	82.60±4.43	85.90±4.25
	Range	(70.00-90.0)	(70.0-90.0)	(75.0-95.0)

Table 2: Mean age of different group (N=150)

Parameters	**Group-A (n=50)**	**Group-B1 (n=50)**	**Group-B2 (n=50)**	**Verses**	**P-value**
Age I years	**Healthy People**	**(HbA1c <6.5%)**	**(HbA1c≥6.5%)**		
	n(%)	**n(%)/**	**n(%)**		
	Mean ±SD	**Mean ±SD**	**Mean ±SD**		
	45.00±5.17	45.96±5.26	45.94±5.29		
				A vs B_1 vs B_2	0.579^{ns}
				A vs B_1	0.359^{ns}
				A vs B_2	0.371^{ns}
				B_1 vs B_2	0.985^{ns}

Table 3: Study parameters of different groups (N=150)

Parameters	**Group-A (n=50)**	**Group-B1 (n=50)**	**Group-B2 (n=50)**	**Verses**	**P-value**
	Healthy People	**(HbA1c <6.5%)**	**(HbA1c≥6.5%)**		
	n(%)	**n(%)/**	**n(%)**		
	Mean ±SD	**Mean ±SD**	**Mean ±SD**		
Fasting Plasma Glucose (mmol/L)	4.31±0.52	6.06±0.98	10.45±3.74		
				A vs B_1 vs B_2	<0.001
				A vs B_1	<0.001
				A vs B_2	<0.001
				B_1 vs B_2	<0.001
HbA_1c (%)	4.70±0.46	6.08±0.33	9.71±2.41	A vs B_1 vs B_2	<0.001
				A vs B_1	<0.001
				A vs B_2	<0.001
				B_1 vs B_2	<0.001
ADA (U/L)	10.48±2.22	17.75±2.25	30.58±6.73	A vs B_1 vs B_2	<0.001
				A vs B_1	<0.001
				A vs B_2	<0.001
				B_1 vs B_2	<0.001

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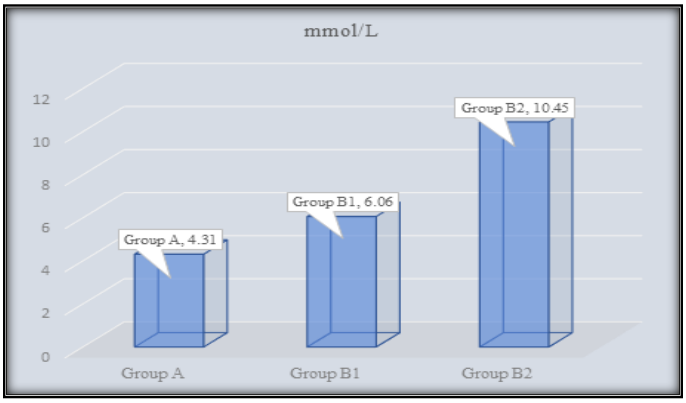

Figure 1: Mean fasting plasma glucose level of the participants (N=150)

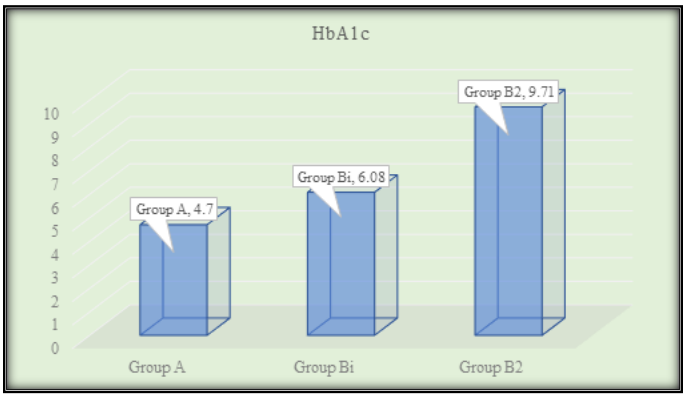

Figure 2: Mean HbA1c level of the participants (N=150)

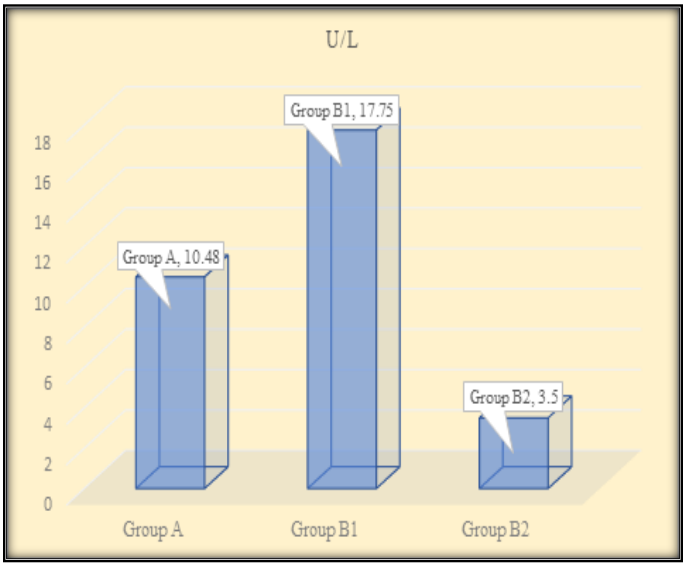

Figure 3: Mean Serum Adenosine deaminase (ADA) level of the participants (N=150)

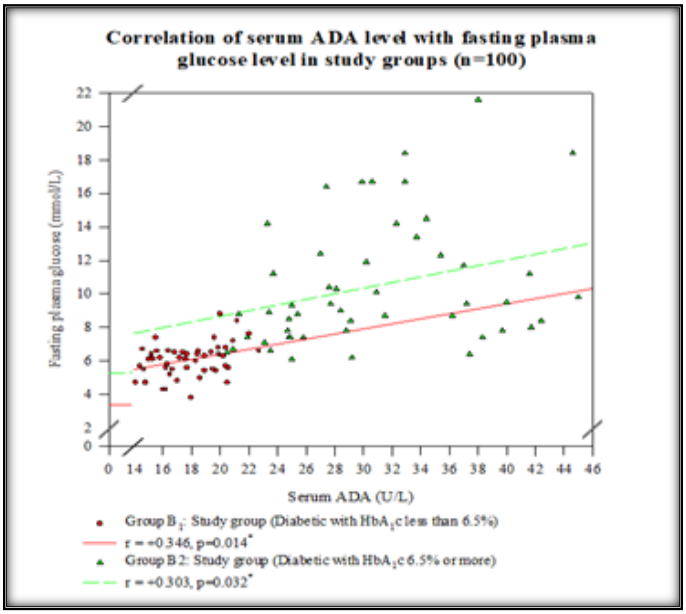

Figure 4: Correlation of serum ADA Level with Fasting plasma glucose

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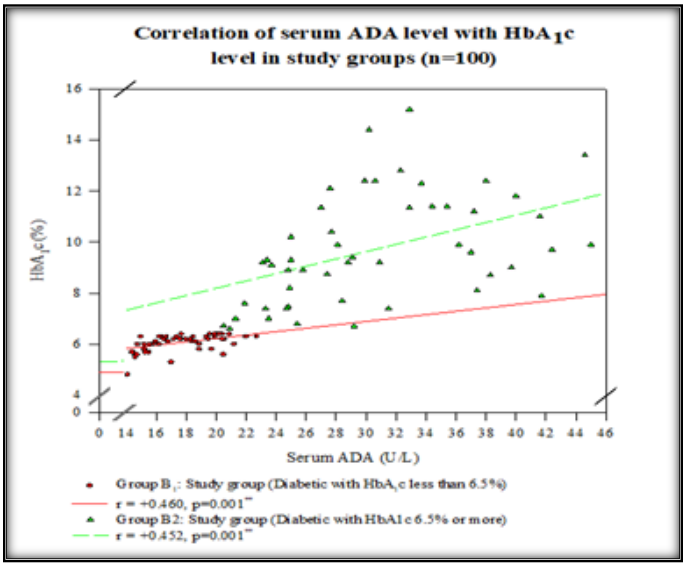

Figure 4: Correlation of serum ADA Level with HbA1c

DISCUSSION

The aim of this study was to observe serum adenosine deaminase (ADA), fasting plasma glucose (FPG) level, HbA1c level. For this study, a total number of 100 type 2 diabetic patients (50 T2DM with HbA1c <6.5% and50 T2DM with HbA1c%≥6.5%) of both sex with age ranging from 40 to 55 years were considered as study group. Again, 50 apparently healthy subjects of both sexes with same age range were included in control group for comparison.[18] In this study, among total 150 participants, male 24 and female 26 diabetic patients with HbA1c<6.5% and male 27 and female 23 diabetic patients with HbA1c≥6.5% were selected for study group. Again, male 23 and female 27 were selected for control group.[19] The mean fasting plasma glucose (FPG) were significantly (p<0.001) higher in control and uncontrolled type-2 diabetes in comparison to that of control group. Again, these values were significantly (p<0.001) higher in patients with uncontrolled type-2 DM than that of control DM. This finding was in agreement with the studies of many researchers of different countries.[20,21] In this study, the mean HbA1c level was also significantly higher in patients with control and uncontrolled type-2 DM as compared to healthy control (p<0.001). Again, the mean HbA1c level was significantly (p<0.001) higher in patients with uncontrolled type-2 DM than that of control type2DM. Almost similar types of results were found by different researchers of different countries.[22] In our study, the mean serum ADA level was higher in patients with control and uncontrolled type-2 DM as compared to healthy people and the result was statistically significant (p<0.001). The mean ADA level was also significantly (p<0.001) higher in patients with uncontrolled type-2 DM than that of control DM. Almost similar to the findings were observed by the various investigators from different countries.[15] This finding was in agreement with the study of many researchers of different countries.[23] In the present study, among the 50 control diabetes patients, 7(14.0%) patient have serum ADA level ≤15.0U/L and rest 43(86.0%) patients have >15.0 U/L. Again, in patients with uncontrolled (n=50), serum ADA level>15.0 U/L were found in 50(100%) patient. In the present study, among the 50 control diabetes patients, but no published data are available in our countries as well as different countries to compare these findings. In the present study, Pearson's correlation coefficient (r) test was done to observe the relationship of serum Adenosine deaminase (ADA) in study groups. In patients with control and uncontrolled DM, ADA and the relationship was statistically significant. Similarly, serum ADA level showed positive correlation with HbA1c in patients

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with control and uncontrolled diabetes and the relationship was statistically significant. Kaur et al.[16] Patel et al.[20] also found highly significant positive correlation of serum ADA HbA1c level. In this study, Pearson's correlation coefficient (r) test was done to observe the relationship of serum ADA in study group. Sahema et al.[21] also found highly significant positive correlation of serum ADA level in T2DM.

CONCLUSIONS

As per the findings of this study we can conclude that, serum ADA level is significantly higher in patients with T2DM which was more in uncontrolled DM than control DM. Increased serum ADA level is positively correlated with glycemic status. Therefore, estimation of serum ADA level might be used as a new parameter for prediction of glycemic status in type 2 diabetes mellitus.

Limitations of the Study

Although optimal care had been tried by the researcher in every step of the study but there were some limitations.

Though it was small sample size study, it might not represent the whole country. Though purposive sampling method were followed, so there may be chance of bias which can influence the results. Because of time limitations, in spite of maximum effort by the researcher, the study was conducted with small sample size. So, it may not be adequate represent the total population and a large sample size would have given a better result.

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