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Diagnostic value of Intermediate Monocytes [CD14+CD16+] in Recent Type 1 Diabetes in Children at Zagazig University Hospital

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Abstract

Background:*Type 1 diabetes mellitus (DM1) is caused by autoimmune selective destruction of pancreatic b-cells. As a multifactorial disease, it is caused by a complex combination of genetic and environmental factors triggering autoimmunity. Peripheral blood monocytes are heterogeneous population and until recently were only divided into two subsets based on CD16 expression – CD16 and CD16+. However, minor CD16+ population can be further subdivided into CD14brightCD16+ and CD14dimCD16+ cells. Therefore, there are three distinct subsets of monocytes: the classical CD14brightCD16, the intermediate CD14brightCD16+, and the non-classical CD14dimCD16+. The main objectives of this study were to study the relation between change in intermediate monocyte level and development of recent onset T1D and to determine if level of intermediate monocyte is a predictive factor for poor residual islet cells function determined by level of HA1C, insulin, c-peptide.* ***Methods:*** *This is a case- control study carried out at pediatric endocrinology outpatient clinic and Clinical pathology department of Zagazig university hospital. All studied groups were subjected to full history taking, thorough clinical examination and laboratory investigations included Cell-surface monocyte phenotypic analysis.****Results:****The most common signs and symptoms of presentation were Acidotic breathing. Dry mouth. Polyuria. Polydepsia. Polyphagia. Wt. loss. DKA occurance. Glucosuria and ketonuria . There was highly significant increase in FBS and HBA1C and highly significant decrease in C. peptide in patient group compared to control group.there was significant increase in absolute count of monocytes and non-significant increase regarding neutrophils and lymphocytes counts in patient group compared to control group.There was highly significant increase in the ratio of classical and intermediate monocytes in T1DM group. While the ratio of non-classical monocytes were non significantly increased in T1DM group compared to control group. Type 1 Diabetes Mellitus patients the CD14+/CD16+monocytes (Intermediate subtype) revealed the highest sensitivity and specificity (75%, 100%) among the other monocytes subtypes as the classical (30%,90%) and the Non classical (5%, 95%). There was highly significant positive correlation between intermediate monocytes ratio and FBS, HbA1C.However, there was significant negative correlation between intermediate monocytes ratio and C-peptide.****Conclusion:****The intermediate monocyte population was found to be expanded in pediatric patients with T1DM. As these cells were shown to have pro-*

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inflammatory activity, they are likely to be implicated in the impaired function of β−cells, with deleterious consequences for the development of T1DM..

Key words: *Diagnosis- Intermediate Monocytes [CD14+CD16+]-Type 1 Diabetes.*

I. Introduction:

Type 1 diabetes mellitus (DM1) is caused by autoimmune selective destruction of pancreatic b-cells. As a multifactorial disease, it is caused by a complex combination of genetic and environmental factors triggering autoimmunity [(1)].

Diabetes-related vascular complications share etiological characteristics. Factors recognized as responsible for the pathogenesis of diabetic vasculopathy are: hyperglycemia, hyperlipidemia, growth factors, hormones, and inflammation. Low grade chronic systemic inflammation underlies DM1 and plays a crucial role in the development of late microvascular complications [(2)].

Furthermore, the onset of microangiopathy is associated with infiltration by inflammatory cells, as well as elevated levels of CRP and proinflammatorycytokines . Infiltration by neutrophils and monocytes, firm adhesion of these leukocytes to vascular endothelial cells are one of the earliest events present for many years before overt retinopathy or nephropathy [(3)].

Peripheral blood monocytes are heterogeneous population and until recently were only divided into two subsets based on CD16 expression – CD16 and CD16+. However, minor CD16+ population can be further subdivided into CD14brightCD16+ and CD14dimCD16+ cells. Therefore, there are three distinct subsets of monocytes: the classical CD14brightCD16, the intermediate CD14brightCD16+, and the non-classical CD14dimCD16+ [(4)].

The marked expansion in intermediate monocytes in patients with T1DM were demonstrated to produce more tumor necrosis factor-alpha (TNF-α), this factor is an effective inflammatory factor, and has been correlated with the severity of T1DM. There is evidence that the intermediate monocytes have an antigen-presenting function with a dendritic cell-like feature. Upon antigen stimulation, studies prove that intermediate monocytes become the main producers of inflammatory factors, like interleukin (IL)-1α, IL-6, and TNF-α [(5)].

The main objectives of this study were to study the relation between change in intermediate monocyte level and development of recent onset T1D and to determine if level of intermediate monocyte is a predictive factor for poor residual islet cells function determined by level of HA1C, insulin, c-peptide.

II. Patients and Methods

Technical design:

- **Study design:**

- The current study is a case-control study that was performed in Pediatric endocrinology outpatient clinic of Zagazig university hospital and Clinical pathology department of Zagazig University in Sharkia Governorate during period from March 2019 to July 2019.

- The study protocol was approved by Ethics Committee, Faculty of Medicine and Zagazig University and was conducted in accordance with the university laws of human researches.

Sample size

- Assuming that mean ± SD of CD14+CD16+ monocyte in T1DM and in control group was (2.38±2.2 versus 6.87±6.7 respectively) So, sample size was calculated by Open Epi program to be 40 cases (20 in each group) with confidence level 95% and power of test 80%.

Subjects:

This study was conducted on 40 children divided into 2 groups:

1-Group I (T1DM group):

20 children having recent onset T1DM

2-**Group II (control group):**

20 age and sex matched healthy children .

Inclusion criteria:-

- Children with recent onset T1DM
- Duration of illness 1-3 months
- Age between 5 years -15 years
- Both sex.

Exclusion criteria:-

- Age < 5 years and > 15 years
- Duration of illness > 3 months
- Patient with micro vascular complications
- Patient with coexisting autoimmune, chronic, and acute inflammatory diseases

Methods:

All the studied groups were subjected to the following:

- ***Complete history taking with special emphasis on:***

- History of present illness (onset of diabetes, duration of symptoms, severity of the disease).
- Past history of (preceding viral illness as mumps or varicella, medication taken, any complication as DKA).
- Family history of DM.

- **Clinical examination with special emphasis on:**

1-General examination:

General appearance with specific attention to abnormalities of growth and with stress on signs of dehydration, dry mouth, acidotic breathing, vomiting, abdominal pain.

2-Vital signs: as Heart rate, respiratory rate and **Anthropometric measures** which included: Weight, height, head circumference, midupper arm circumference**.**

Investigations:

Laboratory investigations

- **Routine laboratory investigations**

As:-

- Complete blood count
- Blood sugar level and ketones in blood
- Blood gases for assessment of acid-base status
- The serum HbA1c level.
- C-peptide levels

- **Special laboratory investigations**

Immunophenotyping of peripheral blood specimen on monocytes population:

Cell-surface monocyte phenotypic analysis was performed after staining with human anti-CD14 and anti-CD16 by flow cytometry

Administrative considerations

- Approval from ethical committee in the faculty of medicine (Institutional Research BoardIRB)

- Informed Consents from the parents, after explanation of the nature and the purpose of the investigations to the parents prior to participation in the study.

Statistical Analysis

Data entry, processing and statistical analysis was carried out using MedCalc ver. 18.2.1 (MedCalc, Ostend, Belgium). Tests of significance (Kruskal-Wallis, Wilcoxon's, Chi square, logistic regression analysis, and Spearman's correlation) were used. Roc CURVE.Data were presented and suitable analysis was done according to the type of data (parametric and non-parametric) obtained for each variable. P-values less than 0.05 (5%) was considered to be statisticallysignificant.

III. Results:

– **Figure (1) and (2)** show non-significant difference between the studied groups regarding age, sex and anthropometric measurements (Wt. and Ht)

– This table shows that the most common signs and symptoms of presentation were Dry mouth, Polyuria, Polydepsia, Polyphagia and Weight loss followed by DKA occurance, Acidotic breathing, Glucosuria and ketonuria . Duration of illness of patients with T1DM was 1.9±0.9 months**(Table 1)**.

– This figure shows highly significant increase in FBS and HBA1C and highly significant decrease in C. peptide in patient group compared to control group**(Figure 3).**

– This table shows significant increase in absolute count of monocytes and non-significant increase of neutrophils and lymphocytes counts in patient group compared to control group**(Table 2)**.

– This table shows highly significant increase in the ratio of classical and intermediate monocytes in T1DM group, While the ratio of non-classical monocytes was non significantly increased in T1DM group compared to control group**(Table 3)**.

– This tables shows that in type 1 Diabetes Mellitus patients the CD14+/CD16+monocytes (Intermediate subtype) revealed the highest sensitivity and specificity (75%, 100%) among the other monocytes subtypes as the classical (30%,90%) and the Non classical (5%, 95%)**(Table 4)**.

– This table shows highly significant positive correlation between intermediate monocytes ratio and FBS and HbA1C. However,there was significant negative correlation between intermediate monocytes ratio and C-peptide**(Table 5)**.

– This table shows significant positive correlation between intermediate monocytes ratio and the clinical parameters which were more common presented in T1DM group as (acidotic breathing, polyuria, polydipsia, polyphagia, dry mouth, weight loss, DKA occurance, glucosuria and ketonuria)**(Table 6)**.

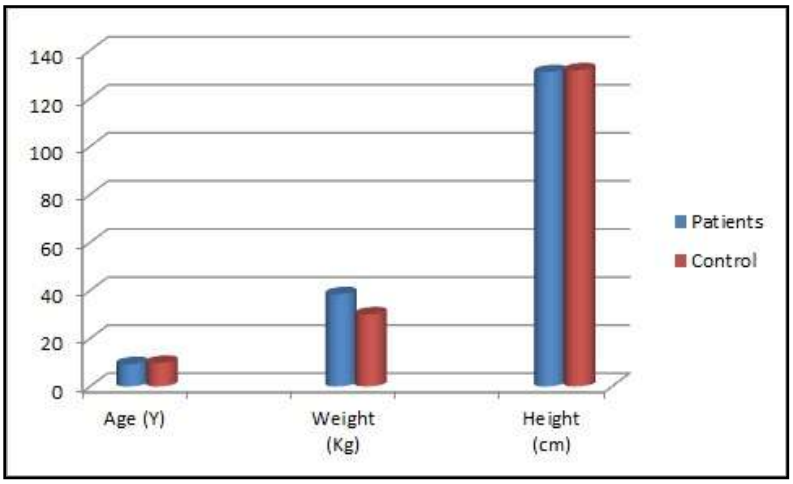


Figure 1: Demographic data of the studied groups.

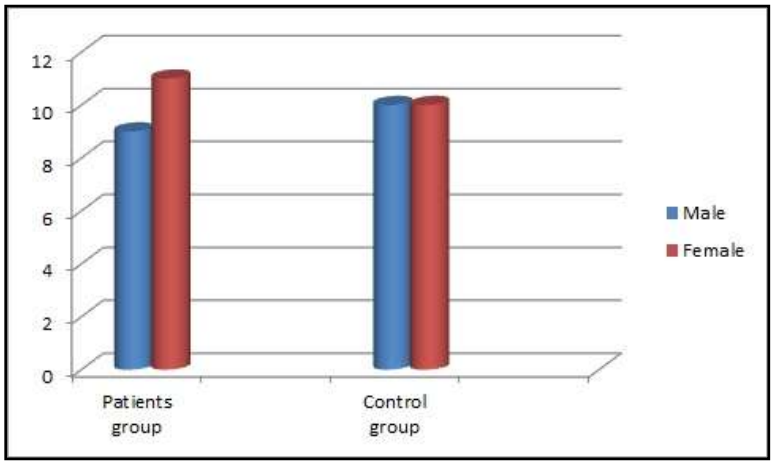
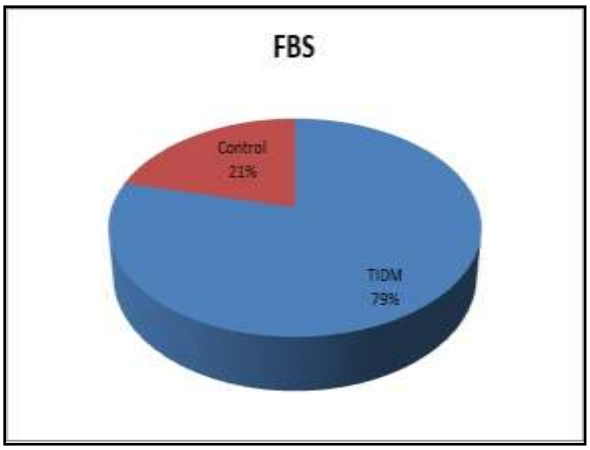


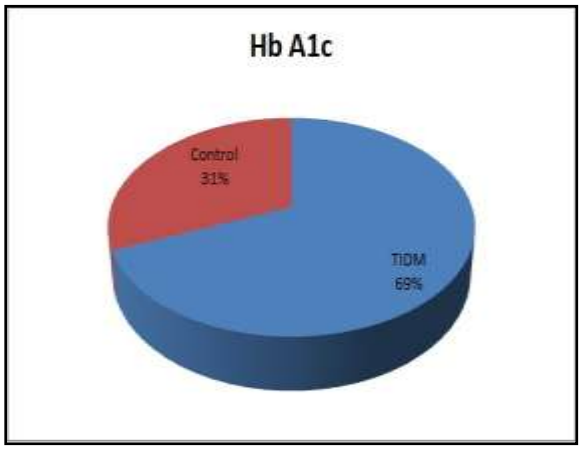
Figure 2: Distribution of gender among the study groups

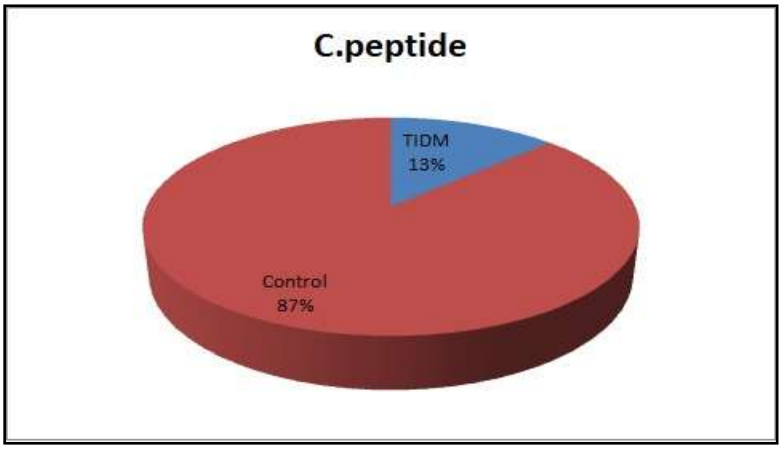
Table 1: Clinical data of patients with type 1 diabetes mellitus.

Clinical Data	**Group I T1DM n: 20**	
	+ve	**-ve**
Family history of DM	**18**	**2**
Preceding viral infection	**9**	**11**
Acidotic breathing	**18**	**2**
Dry mouth	**19**	**1**
Abd. Pain, vomiting	**10**	**10**
Fever	**8**	**12**
Polyuria, polydepsia, polyphagia	**19**	**1**
Weight loss	**19**	**1**
DKA occurance	**18**	**2**
Glucosuria, Ketonuria	**17**	**3**
Duration of illness (Month)	1.9±0.9 (1-3.5)	

DKA: diabetic ketoacidosis







T1DM: type 1 diabetes mellitus

FBS: fasting blood suger

HbA1c: glycatedhaemoglobin

Figure 3: Comparison between patients with type 1 diabetes and Control as regard the levels of FBS, HbA1c and C.peptide respectively.

Table 2: Differential WBCs of CBC of the studied groups.

	Group I TIDM n: 20	**Control group n: 20**	**Student 't' Test**	**P-value**
Monocytes(absolute count)	3.5±0.5 (2.5-4.5)	2.04±0.3 (1.3-2.9)	2.503	0.01*
Neutrophils(absolute count)	1.6±0.4 (0.9-2.2)	1.2±0.4 (0.24-1.94)	0.707	0.483
Lymphocytes(absolute count)	1.9±0.3 (15-2.5)	1.5±0.5 (0.7-2.9)	0.686	0.496

*p>0.05= not significant; *p<0.05=: Significance;*

Table 3: Monocyte subsets characterization in the studied groups

	Group I TIDM n: 20	**Control group n: 20**	**Test of significance**	**P-value**
CD14++/CD16- (classical)	89.4±3.6 (79.8-93.3)	74.9±12.3 (44.9-85.8)	t= 5.059	0.0001*
CD14+/CD16+ (intermediate)	18.5±12.2 (7.8-48)	4.5±1.9 (2.3-8.5)	t= 5.070	0.0001*
$CD14^{Dim}$/CD16++ (non classical)	6.6±1.5 (2.3-10.9)	5.8±1.6 (4.2-10.7)	t=1.631	0.111

*t: student t test; *p<0.05=: Significance*

Table 4: The Sensitivity, Specificity of Monocyte Subsets in T1DM Patients

	Sensitivity	**Specificity**	**PPV**	**NPV**	**Positive Likelihood Ratio**	**Negative Likelihood Ratio**	**Accuracy**
CD14++/CD16- Classical	30%	90%	75%	56.3%	3	0.78	62.5%
CD14+/CD16+ Intermediate	75%	100%	100%	80%	-	0.25	87.5%
$CD14^{Dim}$/CD16++ Non classical	5%	95%	50%	50%	1	1	50%

PPV: Positive predictive value; NPV; Negative predictive value

Table 5:**Correlations between Laboratory parameters and circulating Monocytes subsets cells in patients with type 1 diabetes**

	CD14++/CD16- Classical		CD14+/CD16+ Intermediate		$CD14^{Dim}$/CD16++ Non classical	
	r	P	r	P	r	P
FBS	0.361	0.117	0.452	004*	-0.145	0.541
Hb A_{1c}	0.146	0.537	0.760	0.0001*	0.108	0.649
C. peptide	0.183	0.439	-0.477	0.03*	-0.103	0.665

*p>0.05= not significant; *p<0.05=: Significance*

Table 6: Correlations between Clinical parameters and circulating Monocytes subsets cells in patients with type 1 diabetes

	CD14++/CD16- Classical		**CD14+/CD16+ Intermediate**		**$CD14^{Dim}$/CD16++ Non classical**	
	r	P	r	P	r	P

Acidotic breathing	-0.052	0.827	0.132	0.048	0.003	0.989
Dry mouth	-0.019	0.936	-0.296	0.025	-0.112	0.638
Abd. Pain, vomiting	0.150	0.527	0.163	0.051	-0.416	0.068
Fever	-0.146	0.539	0.090	0.075	-0.423	0.063
Polyuria,polydepsia,polyphagia	0.216	0.360	0.115	0.039	-0.002	0.993
Weight loss	0.199	0.401	0.165	0.044	0.372	0.106
DKA occurance	-0.064	0.788	0.168	0.047	0.274	0.242
Glucosuria, Ketonuria	-0.533	0.155	0.286	0.021	0.403	0.077

*$p>0.05$= not significant; *$p<0.05$=: Significanc*

IV. Discussion

This study was conducted in 40 participants divided into two groups, 20 patients with T1DM, 9 were males(45%) and 11 were females(55%) with mean age of 9.3±2.9 years and a control group including 20 healthy children , 10 were males(50%) and 10 were females(50%) with mean age 9.8±3 years

This study shows that there was non-significant difference between the studied groups regarding age, sex and anthropometric measurements (Weight and Height).

The present study showed that the most common signs and symptoms of presentation were Acidotic breathing. Dry mouth.Polyuria.Polydepsia.Polyphagia.Wt. loss.DKA occurance.Glucosuria and ketonuria . And duration of illness of patients with T1DM was 1.9±0.9 months, There was highly significant increase in FBS and HBA1C and highly significant decrease in C. peptide in patient group compared to control group.

Furthermore, **Renet al.,**[6] observed that Serum HbA1c levels, were significantly higher in patients with diabetes than in controls (p=0.000).

The current study shows that there was significant increase in absolute count of monocytes and non-significant increase regarding neutrophils and lymphocytes counts in patient group compared to control group.

This is consistent with data showing that CD16+ subsets of monocytes are expanded in some autoimmune diseases and may be involved in the induction of inflammatory immune response (7).

In this study, there was highly significant increase in the ratio of classical and intermediate monocytes in T1DM group. While the ratio of non-classical monocytes were non significantly increased in T1DM group compared to control group.

Ryba-Stanisławowskaet al.,[8], showed higher level of CD16+ non-classical and intermediate monocytes in comparison to their healthy counterparts.

The present study shows that in type 1 Diabetes Mellitus patients the CD14+/CD16+monocytes (Intermediate subtype) revealed the highest sensitivity and specificity (75%, 100%) among the other monocytes subtypes as the classical (30%,90%) and the Non classical (5%, 95%).

The results of the present study are in agreement with study of **Renet al.,**[6]as they reported that all of the T1DM patients recruited in this study showed a dramatic increase in HbA1c (11.8% ± 2.5, reference value 4.0–6.0%), and a significant decrease in both the serum insulin concentration (3.2 ± 2.3 IU/ml, reference value 6.0–27 IU/ml) and serum C-peptide level (0.9 ± 0.7 ng/ml, reference value 1.1–5.0 ng/ml) .

The data of the present study showed that there was highly significant positive correlation between intermediate monocytes ratio and FBS, HbA1C.However, there was significant negative correlation between intermediate monocytes ratio and C-peptide.This data agrees with **Ryba-Stanisławowskaet al.,**[8]who observed thatincreased level of HbA1c correlated with the frequency of intermediate and non-classical subsets of CD16+ monocytes. The stronger correlation was seen between HbA1c level and percentage of nonclassical CD14dimCD16+ cells and this is not consistent with our results.

The current study shows that there was highly significant positive correlation between intermediate monocytes ratio and the clinical parameters which were more common presented in T1DM group as (acidotic breathing, Dry mouth, Polyuria, polydipsia, polyphagia, Weight loss. DKA occurance, Glucosuria and Ketonuria).

The levels of insulin and C-peptide are reliable clinical indicators for the competent function of β−cell ,**Dereke et al.,**[9] demonstrated that the absolute number of intermediate monocytes was negatively correlated with the concentrations of C-peptide and insulin. Collectively, these results suggest that expanded intermediate monocytes might play a detrimental role for β−cell function in children with T1DM.

It is possible that the expansion of intermediate monocytes would result in the release of more proinflammatory cytokines, which would eventually lead to a detrimental effect on β−cells. It has been documented that continuous autocrine TNF-α stimulation of CD14+ monocytes drives the cells to differentiate into dendritic cells, which in turn incessantly produce TNF-α and other inflammatory factors [10].

V. Conclusion:

The intermediate monocyte population was found to be expanded in pediatric patients with T1DM. As these cells were shown to have pro-inflammatory activity, they are likely to be implicated in the impaired function of β−cells, with deleterious consequences for the development of T1DM..

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