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Research Article

Impact of blood monocytes (CD14 and CD16) in diagnosis and prognosis of recent onset TIDM

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Abstract

Background: Monocytes have pro-inflammatory role; they are likely to be involved in degradation of β-cells. **Objectives:** To establish the level of intermediate monocyte CD 14+ and CD16+ in patient newly diagnosed T1DM and its impact on the diagnosis and prognosis of T1DM. **Subjects and methods:** This study was analytical cross-sectional study assessed at Pediatric Endocrinology Outpatient, Minia university hospital, 65 children were separated into two groups for the study: Group I (T1DM group) consisted of 40 children with recently developed T1DM, and Group II (control group) consisted of 25 children. The subjects under study underwent thorough history-taking, clinical examinations, and laboratory tests such as FBG, C-peptide, HbA1c, Insulin antibodies, CBC, and level of CD14+ & CD16+. **Ethical Approval**: The ethical council for the faculty of medicine approved this study, and all measures were followed in line with the rules and legislation that applicable. All subjects provided written informed permission, and the Research Ethical Committee authorized the study**. Approval No.277:1/2022 Results:** Our results revealed that the proportionate of intermediate monocytes CD14+, CD16+ were considerably more in cases group compared to control group. HbA1C, RBG, 2-hr postprandial glucose, TTG IgA, IgG had markedly greater levels in the cases group in comparison to the control group. In contrast, C-peptide level was much lower in the cases group compared to the control group. Anti-GAD antibodies were reported in 31 children (77.5%) while IAA were reported in 25 children (62.5%). **Conclusion**: In children with T1DM, the intermediate monocytes are greatly increased and contributes an essential part in the disease's etiology.

Keywords: Children, Type 1 Diabetes Mellitus, Intermediate Monocytes, β-cells.

Introduction

According to ASPAD, type 1 diabetes, also known as T1DM, is a metabolic condition carried on by an immune-mediated assault on pancreatic beta-cells, a process where autoreactive T cells play a critical role[1]. Islet autoantibodies were the most distinctive feature. resulting in hyperglycemia that necessitates ongoing insulin replacement therapy[2]. Autoimmunity is believed to have an essential function in the pathogenesis of T1DM. A viral infection may promote the formation of antibodies against a viral protein in a person with a genetic predisposition, which sets off an autoimmune reaction against antigenically related beta cell components[3]. Monocytes contribute to inflammation both directly and indirectly by dispersing into macrophages and dendritic cells[4]. Human monocytes are divided into 3 main subsets;

classical, non-classical and intermediate, by expression of special surface markers and by their actions in homeostasis and disorders[5].

Intermediate monocytes have been dramatically increased in inflammatory and autoimmune disorders, such as type 2 diabetes, active rheumatoid arthritis, ongoing renal disease, and coronary artery disease. This happens because they perform crucial roles in antigen presentation and cytokine generation. It was shown that intermediate monocyte production of the potent inflammatory cytokine tumor necrosis factor-alpha (TNF-alpha) was significantly higher in those with juvenile-onset T1DM. TNF has been shown to be associated with the progression of T1DM. Actually, with antigen stimulation, intermediate monocytes remain the primary makers of pro-inflammatory molecules, especially interleukin (IL)-1, IL-6, and TNF[6].

Study design: This study was evaluated at Pediatric Endocrinology Outpatient Clinic, Minia University of Children, Obstetrics and Gynecology Hospital over the period from April 2022 to April 2023.

Inclusion criteria: - Children with newly diagnosed T1DM, both sexes, disease lasting 1-3 months, between the ages of 5 and 15 were involved.

Exclusion criteria: - Ages younger than 5 and older than 15 years, illness duration of more than three months, concurrent autoimmune disorders, chronic and acute inflammatory diseases, use of steroids or other medications that impact blood cell count, and T1 or T2 diabetes are all excluded. A thorough clinical examination and meticulous data collection were performed on all study groups. Seven ml of venous blood was withdrawn from every subject by sterile venipuncture for laboratory investigation Two ml in ethylene diamine tetra acetic acid (EDTA) tube for CBC and HbA1c, One ml in EDTA tube for flowcytometry technique. Four ml were let to be clotted in a plain tube for thirty minutes then centrifuged for fifteen minutes at 3000 g then serum was used for determination of Glucose, C-peptide, TTG IgA, TTG IgG, Anti-GAD antibody, Insulin autoantibodies, TSH and FT4. CBC measured using Sysmex diagnostic, USA. HB A1c (Stanbio, Boerne, Texas). We determined chemistry tests, using SAL 6000 modular system fully automated machine, C-peptide, TSH, free T4 was measured using Cobase 411 fully automated Electro Chemiluminiscence Analyser. Anti-GAD antibodies (from RSR, United Kingdom). Insulin antibodies (Demeditec Insulin Ab ELISA, DE7430, Germany), TTG IgA and TTG IgG (Eagle Biosciences, Inc) using Solid Phase Sandwich ELISA. In addition, immune-phenotyping for cell-surface monocyte phenotypic analysis that was performed after staining with human anti-CD14 and anti-CD16 by flow cytometry. An ethical approval was taken from research ethical council committee of faculty of medicine, Minia University. Each parent gave their assent after being fully apprised.

Statistical analysis:

Statistical Package for Social Sciences (SPSS) application version 23 was used to program, tabulate, and statistically investigate the information gathered. Descriptive statistics were performed for both parametric and non-parametric quantitative information using the mean, standard deviation, minimum and peak of the range, and the median and interquartile range, while they were done for categorical data by number and percentage. Analyses were done for Parametric quantitative data between the two groups using independent sample t test. And Non-parametric quantitative data using Mann Whitney test. Analyses were done for qualitative data using: Chi square test (if the number per cell more than 5) and Fisher Exact test (if the number per cell less than 5). The level of significance was taken at (**P value < 0.05**).

Results

Table (1) demonstrates that there wasn't statistically significant variance among the two studied groups regarding the age (p= 0.196), sex (p= 0.603), weight (p= 0.834), height (p= 0.708) and BMI (p=0.415).

Table (2) shows that HbA1C, RBG, 2-hr postprandial glucose, TTG IgA and TTG IgG were statistically considerably higher in the cases group in contrast to the control group. On the other hand, the C-peptide level was statistically much lower in the cases group alternative to the control group. There was no statistically significant difference between the two groups regarding TSH level, free T4, haemoglobin, WBCs count, platelets count and monocytes count. Anti-GAD antibodies were reported in 31 children (77.5%) while insulin autoantibodies were reported in 25 children (62.5%).

Table (3) shows that CD14, CD16++ (NK) and CD14+/CD16+ were statistically substantially greater in the cases group in comparison to the control group.

Table (1): comparison of the demographic information and physical measurments between cases and control groups

		Groups				Test of significance	P value
		Cases (N=40)		Control (N=25)			
Age (years)		10.55 ± 3.52		11.72 ± 3.49		t = -1.307	0.196
Gender	Male	15	37.5%	11	44%	$\chi 2$ = 0.271	0.603
	Female	25	62.5%	14	56%		
Weight (cm)		37.35 ± 15.14		38.96 ± 13.46		z = -0.209	0.834
Height (kg)		139.93 ± 19.21		141.72 ± 17.85		t = -0.376	0.708
BMI (kg/m^2)		17.93 ± 3.61		18.66 ± 3.26		t = -0.820	0.415

P: probability. Continuous data are expressed as (mean ± SD)
Categorical data expressed as Number (%) $\chi 2$= Chi-square test
T: independent samples t-test z: Mann Whitney U test

Table (2): Comparison between cases and control groups regarding the laboratory parameters

	Groups				Test of significance	P value
	Cases (N=40)		Control (N=25)			
C-peptide (ng/ml)	0.57 ± 0.10		3.35 ± 0.55		t = -31.462	<0.001*
HbA1C (%)	7.72 ± 1.13		5.24 ± 0.60		t = 10.241	<0.001*
RBG (mg/dl)	197.75 ±40.28		108.48 ± 20.26		t = 10.277	<0.001*
2-hr postprandial glucose(mg/dl)	222.95 40.55		151.32 31.15		t = 7.542	<0.001*
TSH (mIU/L)	1.64 ± 0.65		1.72 ± 0.50		z = -1.254	0.249
Free T4 (ug/dl)	1.07 ± 0.12		1.06 ± 0.13		t = 0.514	0.609
TTG IgA(u/ml)	12.57 ± 8.31		2.51 ± 0.99		z = - 5.098	<0.001*
TTG IgG(u/ml)	48.54 ± 26.54		2.61 ± 1.40		z = - 6.742	<0.001*
Anti-GAD(u/ml) ((antibody(u/ml)	31	77.5	0	0%	FET=37.040	<0.001*
Insulin autoantibodies(nU/ml)	25	62.5	0	0	FET=25.391	<0.001*

P: probability. Continuous data are expressed as (mean ± SD)
T: independent samples t-test z: Mann-Whitney u-test
FET= Fischer's exact test *: statistically significant (p< 0.05)

Table (3): Comparison between cases and control groups regarding the CD14 and CD16

	Groups		Test of significance	P value
	Cases (N=40)	Control (N=25)		
CD14(%)	89.42 ± 2.58	72.37 ± 6.55	t = 14.784	<0.001*
CD16++ (NK)	6.53 ± 1.20	5.49 ± 0.75	t = 3.881	<0.001*
CD14+/CD16+	17.78 ± 6.64	4.46 ± 1.15	z = - 6.682	<0.001

P: probability. Continuous data are expressed as (mean ± SD)
T: independent samples t-test z: Mann Whitney U test
*: statistically significant (p< 0.05)

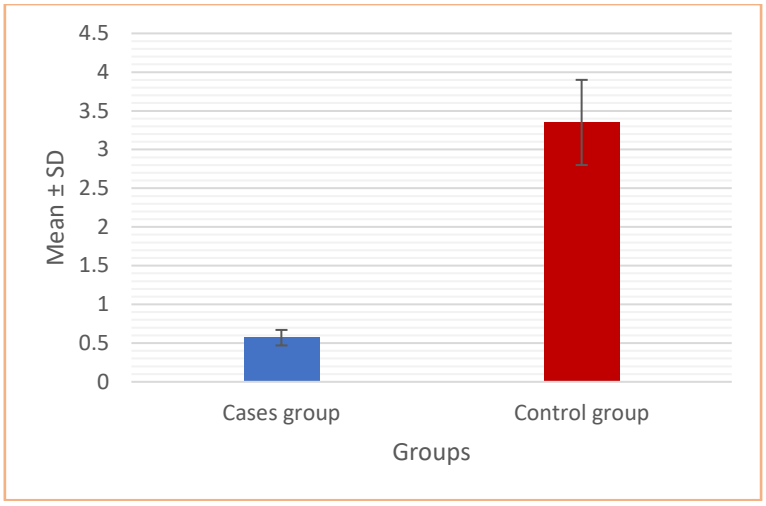

Figure (1): C-peptide in the two study groups

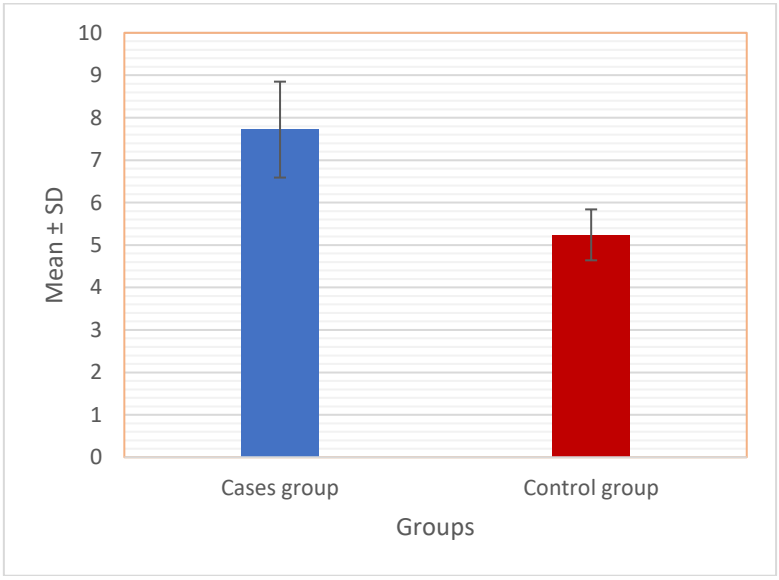

Figure (2): HbA1C (%) in the two study groups

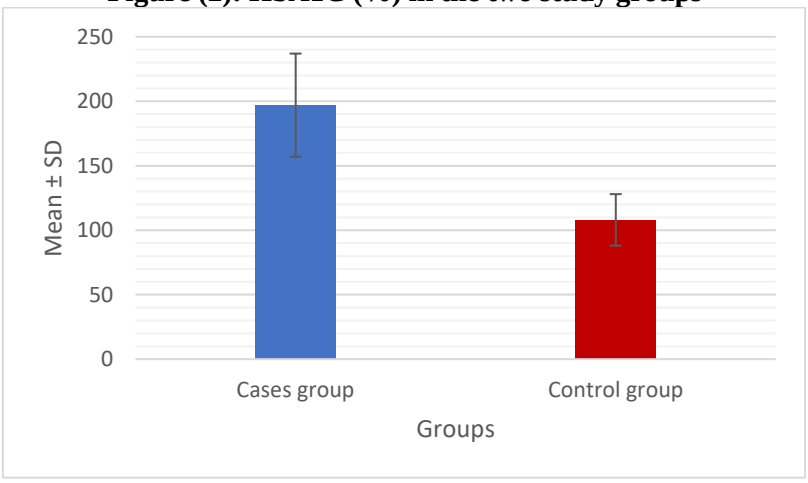

Figure (3): RBG in the two study groups

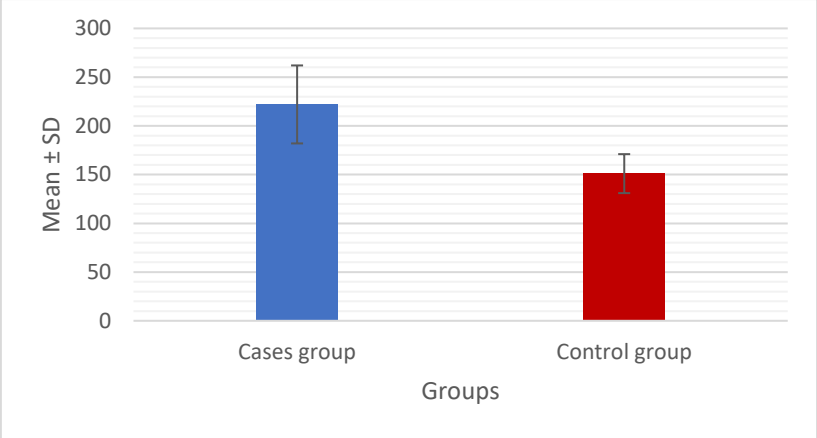

Figure (4): 2-hr postprandial glucose in the two study groups

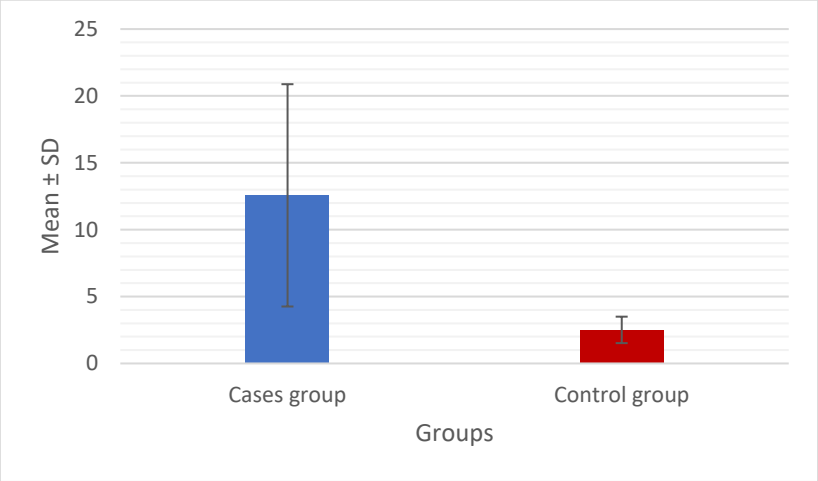

Figure (5): TTG IgA in the two study groups

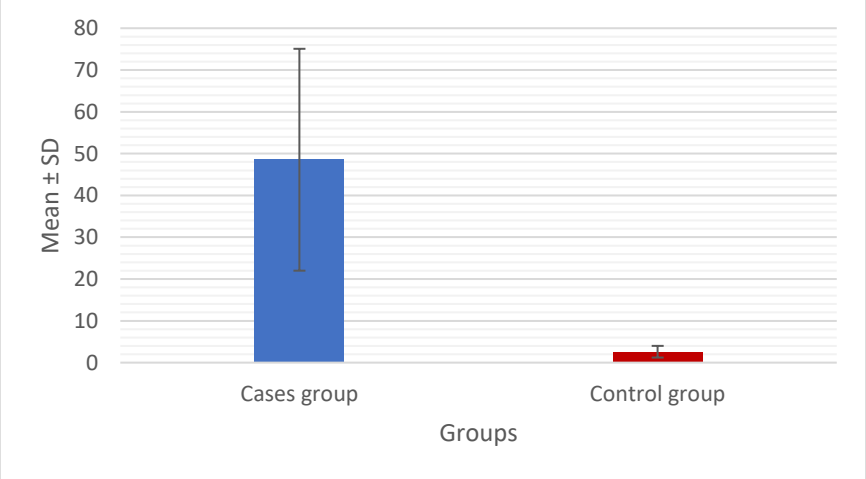

Figure (6): TTG IgG in the two study groups

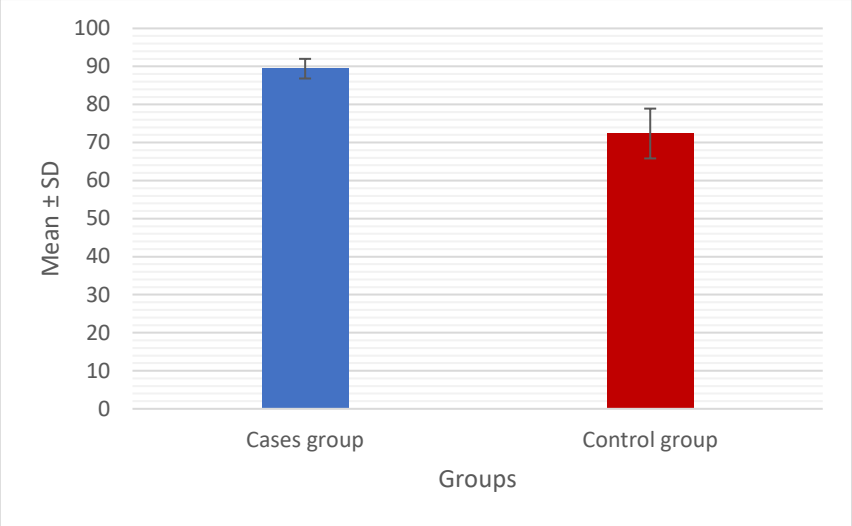

Figure (7): CD14 in the two study groups

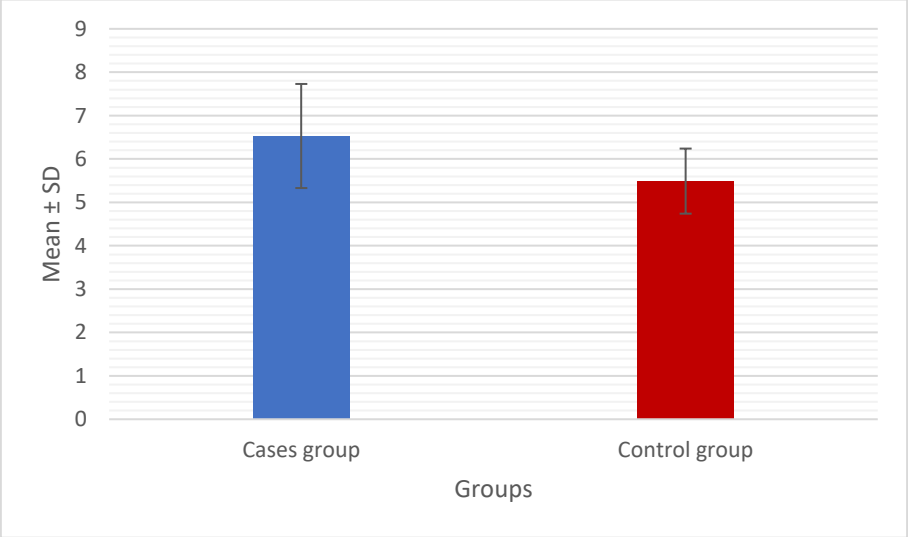

Figure (8): CD16++ (NK) in the two study groups

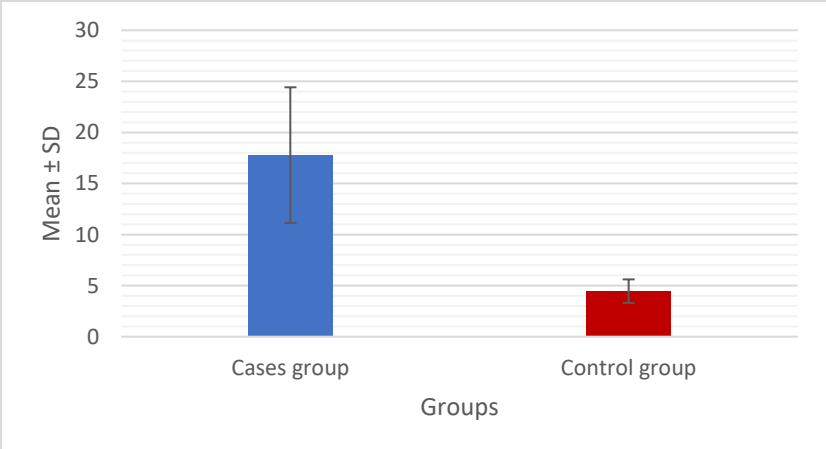

Figure (9): CD14+/CD16+ in the two study groups

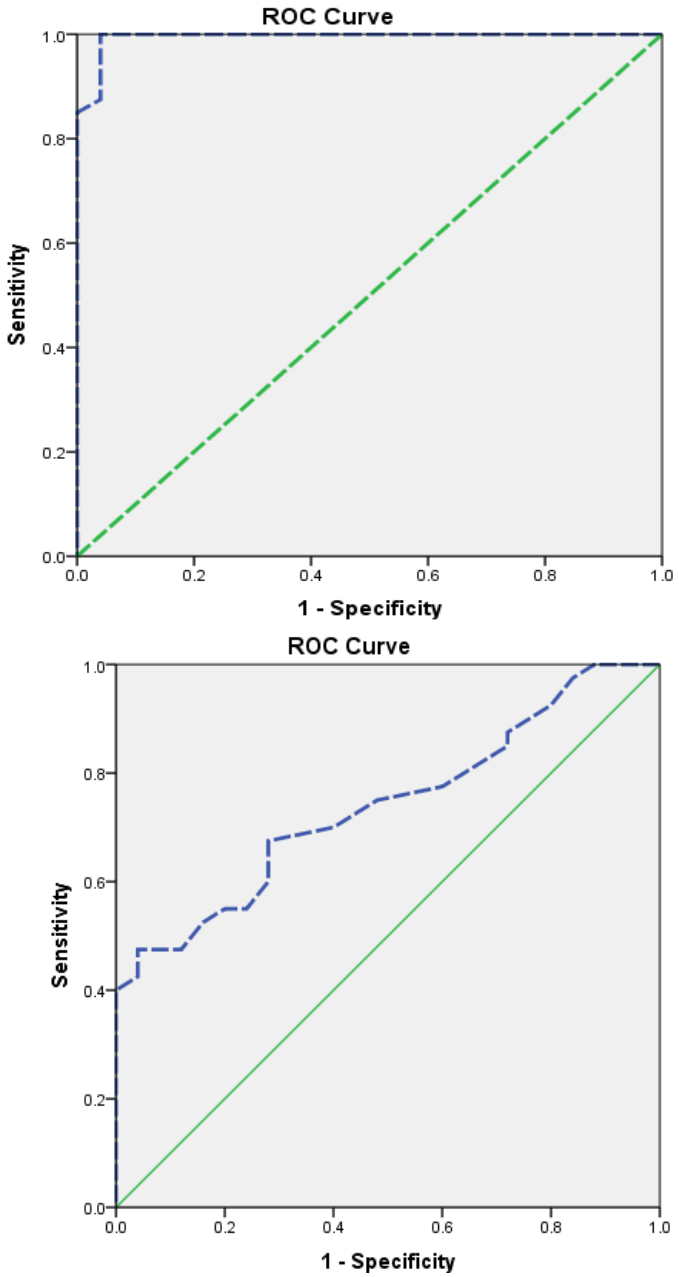

Figure (11): ROC curve of CD16++ (NK) in identifying cases with T1DM

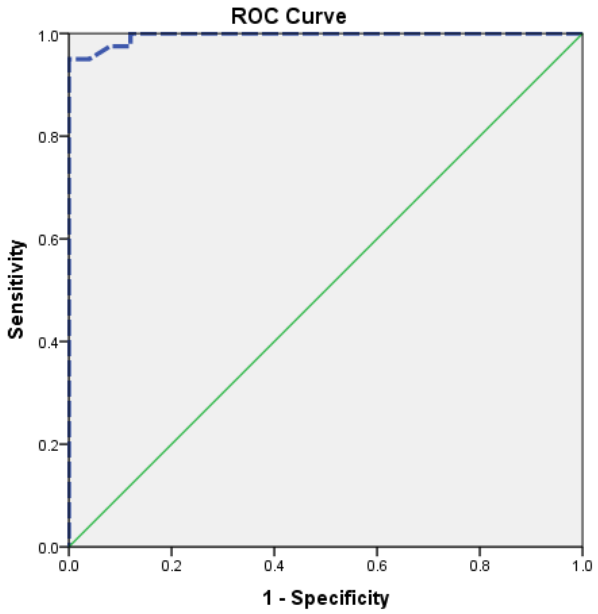

Figure (12): ROC curve of CD14+/CD16+ in identifying cases with T1DM

Discussion

Type 1 diabetes mellitus (T1DM) has been defined by several studies as a persistent, subclinical pro-inflammatory condition characterized by higher circulating pro-inflammatory chemicals started in childhood[7].

Acute and chronic hyperglycemia both boost inflammatory cytokine production and inflammation markers in cultured human blood cells and persons with impaired glucose metabolism[8].

Monocytes are circulating short-lived cells that are involved in inflammation by both direct actions and also through converting into dendritic cells and macrophages. These cells come from the common myeloid progenitor in the bone marrow and are secreted in the bloodstream. They give three major groups named classical (CD14+CD16-), non-classical (CD14-CD16+) and intermediate monocytes (CD14+CD16+) Each of them is differentiated from the other by the expression of special surface markers and by their actions in homeostasis and disorders[4].

This study's major goal was to investigate the relationship between changes in intermediate monocyte levels and the development of T1DM just lately. 65 people, separated into two groups, got involved in this study 40 with T1DM 15 males (37.5%), and 25 females (62.5%) with a mean age of 10.5 ± 3.5 years. the control group included 25 healthy children, 11 were males (44%) and 14 were females (56%) with mean age of 11.72 ± 3.49 years.

In our study, we didn't detect any significant difference among the two studied groups concerning age, sex, weight (37.35 ± 15.14), height (139.93±19.21), and BMI (17.93±3.61). With p-values (0.834, 0.708, 0.415 respectively).

These findings were congruence with[9], who recorded non-significant differences between the studied groups regarding age, sex, and anthropometric measurements (weight, height, and BMI). This explains that our cases with recent onset T1D have no chronic effect on weight, height.

Through our study we noticed that most of diabetic cases initially presented with DKA, it varies from 40:65%, which is supported by [10] who found that diabetic ketoacidosis (DKA) is frequently the first manifestation of childhood T1DM, with a range of 15 to 67%. Also,[11] reported that

in children below three years, more than 50% had DKA as their initial presentation of TIDM and explained this due to the accidental diagnosis of the disease as the patient complaint of acidotic breathing not the classical symptoms as weight loss, polyurea, polydipsia.

In regard to the laboratory parameters of the surveyed children, we observed that the glycated hemoglobin (HbA1C), and 2-hr postprandial glucose were statistically significant in the cases group in contrast to the control group (HBA1C % 7.72 ± 1.13) in case group & (5.24 ± 0.60) in the control group (with P<0.001). While the C-peptide level was statistically significantly lower in the cases group (0.57 ± 0.10 ng/m) in comparison with the control one (3.35 ± 0.55 ng/m)[12] (P<0.001). These outcomes were in agreement with[13], who reported similar results concerning HbA1C (11.3% ± 2.06 in T1DM compared to 5.14% ± 0.2 in control), fasting blood sugar (309.2 mg/dl ± 6.6 in T1DM compared to 82.9 mg/dl ± 7.1 in control), and C-peptide (0.54 ± 0.1 ng/ml in T1DM compared to 3.6 ng/ml ± 0.8 in control).

Regarding antibodies associated with our patients the study concluded that the antibodies in children with Type 1 diabetes based on serology (TTG IgA 12.57 ± 8.31) in case group & (2.51 ± 0.99) in the control group) (TTG IgG 48.54 ± 26.54 in case group &2.61 ± 1.40 in control) although these children were not presented with the classical symptoms of celiac disease as abdominal distension and diarrhea.

Also,[14] found an incidence of serology-positive celiac antibodies in local population of South African children with type 1 diabetes mellitus of 10.2%**.**

We noticed in our study Thyroid function &Thyroid autoantibodies were normal in our diabetic children even those who were discovered with positive CD antibodies and this is also in agreement with[15] study and state that may later on these children may have autoimmune thyroid disease due to the autoimmune base of diabetes.

Though our study Anti-gamadecarboxylase (GAD) antibodies were reported in 31 children (77.5%) while insulin autoantibodies(IAA) were reported in 25 children (62.5%), that's in agreement with the study[16] who also found insulin antibodies abundant in children with T1DM.

According to the current investigation, we noticed that CD14, CD16++ (NK), and CD14+/CD16+ (17.78 ± 6.64 in TIDM compared to 4.46 ± 1.15 in control group) were statistically significantly greater in the cases group compared to the control one. These results were consistent with[17], who found a highly significant increase in the ratio of classical (CD14++/CD16- = 89.4 ± 3.6) and (CD14+/CD16+ = 18.5 ± 12.2) intermediate monocytes in T1DM group. While, the ratio of non-classical monocytes ($CD14^{Dim}$/CD16++ =6.6 ± 1.5) was non-significantly increased in T1DM group compared to the control one.

Conclusion:

It was discovered that children with T1D M had an enlarged population of intermedi -ate monocytes. Considering that these cells have been proven to exhibit proin-flammatory activity, it is probable they are responsible for reduced cell function, which has adverse impacts on the onset of T1DM.

A greater comprehension of the connections between these proinflammatory cells and the degeneration of cells in children with T1DM may result from further characterization of the role of intermediate monocytes.

Recommendation: A greater awareness of the associations between these inflammatory cells and the degeneration of B-cells in children with T1DM may result from additional explanation of the role of intermediate monocytes. Further studies on larger sample size and geographical scale to accentuate our outcome.

Abbreviation: T1DM (type 1 diabetes mellitus), FBG (fasting blood glucose), CD14 (Cluster of differentiation), ASPAD (American Society of adolescent and

pediatric diabetes), TNF (tissue necrotizing factor), DKA (diabetic ketoacidosis), GAD (gamma decarboxylase), IAA (insulin autoantibodies), CD (celiac disease), TTG (tissue trans glutaminase).

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