Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Dendritic cells and monocyte subsets in children with Gaucher disease

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BACKGROUND: There are minimal data on the frequencies of monocyte subsets and dendritic cells (DCs) in children with Gaucher disease (GD), as nearly all previous studies have involved adult patients. Consequently, we aimed to describe the changes in these cell subpopulations in children with GD type 1 who were on regular enzyme replacement therapy (ERT).
METHODS: This case–control study included 25 children with GD1 and 20 healthy controls. All participants underwent investigations such as complete blood count and flow cytometric assessment of DC and monocyte frequencies and phenotype.
RESULTS: We found that GD1 children had significantly reduced percentages of both types of DCs, i.e., plasmacytoid DCs and myeloid DCs, compared to the control group. There was also a significant reduction in absolute monocyte numbers and percentage of classical monocyte. Moreover, the GD1 children had higher frequencies of non-classical and intermediate monocytes than the control group.
CONCLUSIONS: Our results so far indicate that, when compared to the control group, the GD1 children had significantly reduced total and classical monocyte, with significantly decreased frequencies for both types of DCs. These changes can contribute to immunological abnormalities in pediatric patients with GD1.

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IMPACT:

- Children with Gaucher disease type 1 (GD1) have significantly reduced total and classical monocyte frequencies, with decreasing percentages for both types of dendritic cells.
- GD1 children had significantly reduced frequencies of myeloid and plasmacytoid dendritic cells as compared to the controls. The GD1 children also had significant changes in monocyte subsets when compared to the controls.
- Our results show that monocytes and dendritic cells' significant changes could contribute to immunological abnormalities in pediatric patients with GD1.

INTRODUCTION

Gaucher disease (GD) is a hereditary disease caused by deficiency of the lysosomal enzyme β-glucocerebrosidase. Consequently, excess glucosylsphingosine and glucosylceramide accumulate in the bone marrow (BM), lungs, visceral organs, and brain. Early diagnosis of GD is a challenge due to the wide variability in the severity and clinical picture of the disease.[1–3] Besides the usual manifestations of GD, patients have a high susceptibility to bacterial and non-bacterial infections, which may be attributed to the decreased neutrophil count and/or defects in the microbicidal effects of the mononuclear phagocytes.[3–5] Patients with GD often have immune abnormalities that affect either the cellular or humoral immune systems, e.g., reduced numbers of natural killer (NK) cells, cytotoxic, and helper T lymphocytes. Defects in antigen-presenting cells (APCs) have also been reported in GD.[6–8]

The BM produces dendritic cells (DCs), which are considered the most potent professional APCs crucial for priming T lymphocyte's responses.[9,10] DCs are found mainly within tissues and body surfaces; they sense the body for self- and non-self-antigens. They are responsible for taking up and processing antigens, subsequently presenting them to T cells.[9,10] DCs recognize antigens via Toll-like receptors, which are specific for certain molecules present in fungi, bacteria, parasites, and viruses.[9,11] There are two major subsets of DCs: plasmacytoid DCs (pDCs) and conventional or myeloid DCs (cDCs), which are characterized by diverse origins, receptors, and functions. In general, cDCs may be categorized into many subsets, based on their location, function, and phenotype.[9–11] They are potent highly phagocytic APCs in its immature state and highly APC when they are mature, with the capability to secrete enormous amounts of cytokines.[11] pDCs are the second major type of DCs. They are mainly found in lymphoid tissues and not often in non-lymphoid tissue. Upon inflammation, they are rapidly recruited to tissue sites.[11,12] According to pDC maturation status, they have pro-inflammatory and tolerogenic functions. Besides, they express co-

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stimulatory molecules and major histocompatibility complex (MHC II). pDCs also have an antiviral role through the production of interferon (IFN) I and III and the priming $CD8^+$ T lymphocytes and NK cells.[7–10] As a monocyte-derived phagocytic system, DCs express β-glucocerebrosidase and may be affected by the accumulated fat in GD.[4]

Monocytes are BM-derived leukocytes that are able to differentiate into monocyte-derived DCs and monocyte-derived macrophages that regulate both adaptive and innate immune responses. Under basal conditions, monocytes do not proliferate. After stimulation, they migrate to different tissues, express cytokines, and segregate into macrophages or DCs.[13,14] Three monocyte subclasses have been identified based on the expression of the surface markers CD16 and CD14. The classical monocyte subtype represents 85–90% of monocytes and expresses only CD14 and no CD16 (CD14hiCD16−). They are highly phagocytic and scavenger cells.[12] Monocytes expressing CD16 are intermediate and non-classical monocytes. Intermediate monocytes (2–8%) have relatively high CD14 expression levels and some CD16 expression ($CD14^+$/CD14hiCD16). They have many functions, including the manufacturing of reactive oxygen species and angiogenesis.[13,15] Non-classical monocytes (2–11%) have high levels of CD16 and low CD14 (CD14low/CD14−CD16hi). In response to infection, they secrete inflammatory cytokines.[13–15] Both types have a role in antigen presentation and stimulation of T lymphocytes.[13–15]

Data on the frequencies of DCs and monocyte subsets in children with GD are very limited, as nearly all previous studies have involved adult patients. Consequently, we aimed to describe the changes in these subpopulations in children with GD on regular enzyme replacement therapy (ERT).

MATERIALS AND METHODS

All protocols and investigations of our study were following the regulations of the research ethics committee of Assiut University. Written informed consents of parents of all participants were taken.

Study design

This was a case-controlled study undertaken in Assiut University Hospitals, Egypt.

Patients. Our study included 25 children with a confirmed diagnosis of GD type 1 (GD1; age, 3–16 years; 16 boys; 64%). We recruited all patients from the Hematology Unit of Assiut University Children Hospital, Egypt. Twenty age- and sex-matched healthy children were enrolled as controls. All GD children were on regular ERT: 45 IU imiglucerase twice monthly for at least 12 months before the study. We excluded patients with unconfirmed diagnosis, other GD types, and patients with active infections or who had received any immunosuppressive drugs, e.g., steroids.

Methodology. All patients and controls underwent clinical examination for bone abnormalities, neurological manifestations, and any comorbid disorders. All participants underwent investigations such as complete blood count (CBC) by Ruby Cell Dyn (American, Serial number: 36026BG), including white blood cell count, platelet count, hemoglobin, and absolute count of total monocytes, in addition to flow cytometric assessment of DC and monocyte subsets. Five-ml blood samples were collected from all participants into vacutainer tubes containing EDTA for CBC and flow cytometric assessment. The samples were processed within 3 h after collection.

Detection of DCs phenotypes

DCs in blood samples were analyzed by staining 100 µl blood sample with 10 µl fluoroisothiocyanate-conjugated monoclonal antibodies (MoAbs) against lineage markers (including CD3, CD14, CD16, and CD19), allophycocyanin-conjugated CD123, peridinin chlorophyllprotein (Per-CP)-conjugated human leucocyte antigen (HLA)-DR, and phycoerythrin (PE)-conjugated CD11c (all from Becton Dickinson [BD] Biosciences, San Jose, CA). After 30-min incubation at 4 °C in the dark, the red blood cells (RBCs) were lysed. After washing, the cells were resuspended in phosphate-buffered saline (PBS) and analyzed by fluorescence-activated cell sorter flow cytometry (FACSCalibur) with the CellQuest software (BD Biosciences). We analyzed 100,000 events, and isotype-matched negative controls were used with all samples. The number of $CD123^+$ and $CD11c^+$ cells was detected (Fig. 1).

Detection of monocyte subsets

For detecting the monocyte subsets, 100 µl blood sample was stained with 10 µl Per-CP-conjugated CD16 and allophycocyanin-conjugated CD14. All MoAbs were from BD Biosciences. After 30-min incubation at 4 °C in the dark, RBCs were lysed. After one wash, the cells were resuspended in PBS. Flow cytometric analysis was done using a FACSCalibur unit with the CellQuest software (BD Biosciences). About 20,000 events were acquired. The isotype-matched negative control for each sample used anti-human immunoglobulin G (IgG). We used forward and side scatter to define the monocyte populations. Then CD14 and CD16 expression within the monocyte populations were assessed. The method for the absolute count of total monocytes is the CBC cell counter. The classical ($CD14^{++}CD16^-$), intermediate ($CD14^+CD16^+$), and non-classical monocytes ($CD14^-$/dim$CD16^{++}$) were detected and expressed as a percentage of total monocytes (Fig. 2).

Statistical analysis

Data analysis was done by statistical package for social sciences (SPSS), version 17. All data were expressed as the mean and standard deviation of the mean (SD). Differences between the groups were examined for statistical significance using independent sample *T* test. A *p* value of ≤0.05 denoted the presence of a statistically significant difference.

RESULTS

Table 1 shows the clinical and demographic characteristics of the GD1 patients and the controls. Blood cell analysis showed that the GD1 group had a significant decrease in hemoglobin and white blood cells ($p = 0.001$ and 0.005, respectively) and a non-significant decrease in platelet count (Table 2). Regarding the circulating DC subsets in the peripheral blood, the two types of DCs were differentiated according to CD11c and CD123 expression: cDCs ($CD11c^+CD123^-$) and pDCs ($CD11c^-CD123^+$). GD1 children had significant reductions in the percentages of both cDCs (GD: 0.63 ± 0.35% versus control: 1.02 ± 0.34%, $p = 0.001$) and pDCs (GD: 0.12 ± 0.09% versus control: 0.40 ± 0.15%, $p = 0.026$) when compared to the controls (Table 2 and Fig. 3).

Regarding the monocyte subsets, the GD1 group had significantly fewer absolute monocytes (GD: $0.47 \pm 0.30 \times 10^9$ versus control: $0.76 \pm 0.25 \times 10^9$, $p = 0.002$) and had significantly decreased frequency of classical monocytes (GD: 76.79 ± 3.28% versus control: 84.12 ± 2.27%, $p = 0.001$) (Table 2 and Fig. 4). However, GD1 children had significantly increased frequencies of the intermediate ($p = 0.001$) and non-classical monocytes ($p = 0.001$) compared to the control group (Table 2 and Fig. 4).

DISCUSSION

To elucidate the implication of DCs as one of the most potent and specific APCs in children with GD1, we analyzed DCs and their subsets in children with GD1 and in control children. GD patients' peripheral blood had significant reductions in both DC subsets (cDCs, $p = 0.001$; pDCs, $p = 0.026$) compared to the control group. In line with our study, others[5,8,16] have found decreased DCs in adult GD patients. Micheva et al.[5] studied 10 adults and 2 children

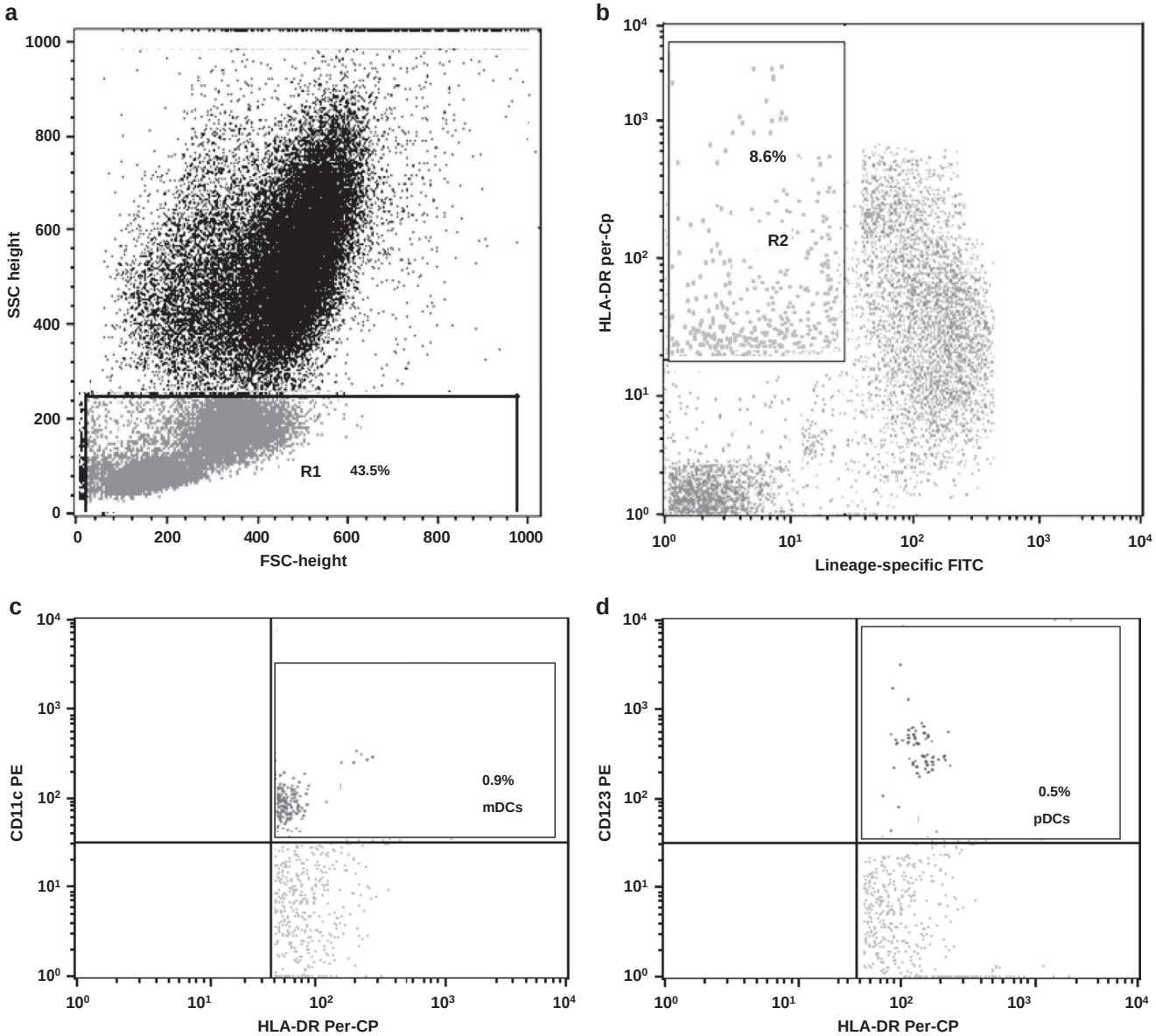

Fig. 1 Representative figure of flow cytometric detection of dendritic cells. a Forward (FSC) and side (SSC) scatters are used to define the lymphocyte and monocyte population (R1). b R2 gate containing entire dendritic cells (HLA-DR$^+$ lineage-specific populations) was selected. c, d Then the percentage of mDCs (HLA-DR$^+$ lineage-specific CD11c$^+$) (R3) and the pDCs (HLA-DR$^+$ lineage-specific CD123$^+$) (R4) were detected.

with GD1 and found a significant reduction in both cDC (p = 0.009) and pDC (p = 0.004) numbers in adult patients. However, the pediatric patients showed comparable frequencies of both types of DCs when compared to healthy volunteers.[5] Functionally, cDCs and pDCs can provoke division of naive T lymphocytes driving T helper type 1 (Th1) and Th2 responses.[17] Moreover, pDCs stimulate Th1 polarization once stimulated by viral infections. These effects are mediated by type I IFN and interleukin-12. Therefore, pDCs comprise a crucial connection between adaptive and innate immunity.[18] Braudeau[8] reported that the absolute numbers of pDCs (p = 0.003) and cDCs (p = 0.018) in 7 adults with GD were significantly reduced as compared to the control group. Moreover, they found a significant reduction in frequencies of γδ2 T cells (p = 0.022) and NK cell numbers (p = 0.043) in GD patients.[8] Interestingly, they found that, compared to the control group, GD patients displayed a significant decline in IFNα-producing pDCs after stimulation with TLR9. They hypothesized that this decline might be a consequence from the disturbance of endosomal signaling pathways of TLR9, which may result from the accumulation of glycosphingolipid in pDCs.[8] This decline returned to normal conditions after ERT. Also, ERT was associated with a decline in pDCs manufacturing the tumor necrosis factor-α cytokine upon triggering with TLR7/8.[8] Sønder et al.[16] observed a highly significant reduction in the number of DCs in splenectomized adult GD patients as compared to healthy controls. However, they found comparable frequencies of cDCs and pDCs among the patients and controls. The reduction in DC frequencies in these reports[5,8,16] and our study could be explained by the increased migration and homing of the peripheral blood DCs to lymphoid tissues due to chronic antigenic stimulation, in addition to the suppression of DC production from pluripotent hematopoietic stem cells in the BM.[5]

Monocytes are a type of leucocyte circulating in the peripheral blood and play a vital role in immunity. They are formed inside the

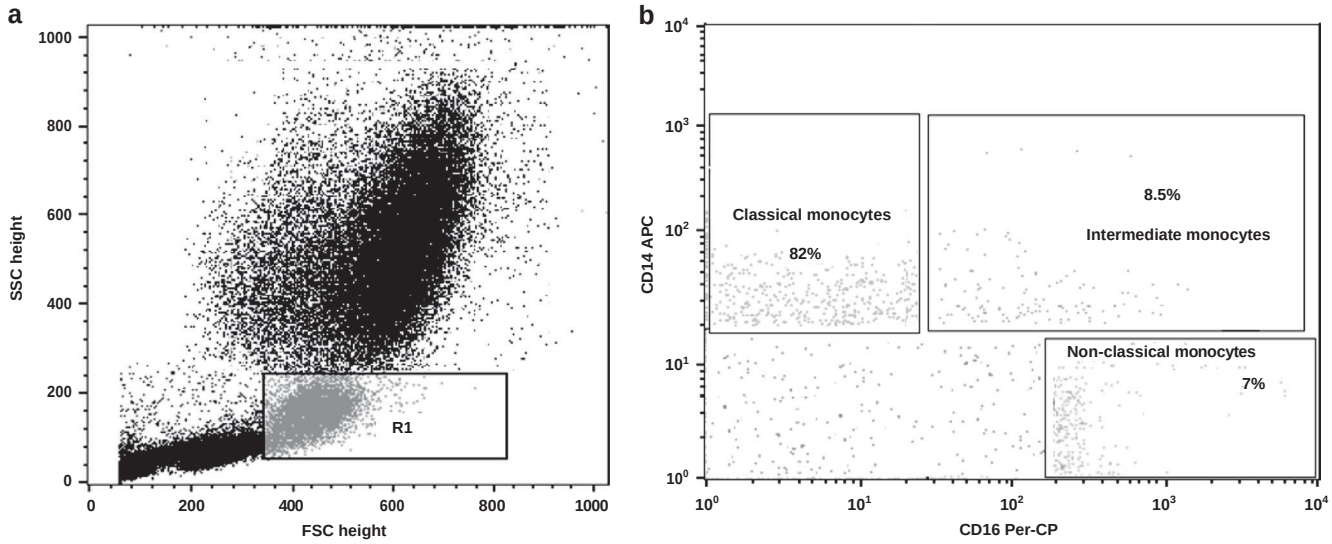

Fig. 2 Representative figure of flow cytometric detection of monocyte subsets. a Forward (FSC) and side (SSC) scatters are used to define the monocyte's population (R1). b The expression of CD14 and CD16 were analyzed on the monocyte's population. Then the classical monocytes, ($CD14^{++}CD16^{-}$), intermediate monocytes ($CD14^{+}CD16^{+}$), and non-classical monocytes ($CD14^{-}$/dim $CD16^{+}$) were detected.

Table1. Demographic and clinical characteristics of patients with Gaucher disease and controls.

	Patients (n = 25)	Controls (n = 20)	p value
Weight (Kg)	34.12 ± 13.46	44.14 ± 14.3	0.039[a]
Age (years)	11.2 ± 2.91	12.04 ± 2.97	0.176[a]
Gender M/F	16/9	12/8	0.26
Liver span/cm (median ± SD)	11.5 ± 3.1	7.9 ± 1.4	0.001[a]
Spleen span/cm (median ± SD)	9.7 ± 5.1	7.3 ± 1.5	0.003[a]
Bone involvement			
Erlenmeyer flask deformity	23/25	—	—
Osteopenia	23/25	—	—
Fracture	2	—	—
Kyphoscoliosis	2/25	—	—
Pigeon chest	2/25	—	—

Data are represented as mean ± SD, $p < 0.05$ is significant.
[a]Independent sample T test.

Table 2. Dendritic cell and monocyte subsets in patients with Gaucher disease and controls.

	Patients (n = 25)	Controls (n = 20)	p value
Hemoglobin, g/dl	10.31 ± .2.17	12.70 ± 2.67	0.001
White blood cells	6.73 ± 1.34	11.91 ± 1.14	0.005
Platelets, 10^9/l	202.05 ± 75.1	234.17 ± 80.4	0.16
mDC % ($CD11c^{+}CD123^{-}$)	0.63 ± 0.35	1.02 ± 0.34	0.001
pDC % ($CD11c^{-}CD123^{+}$)	0.12 ± 0.09	0.40 ± 0.15	0.026
mDC/pDC ratio	2.77 ± 1.05	2.12 ± 0.58	0.43
Absolute monocytes ($\times 10^9$)	0.47 ± .30	0.76 ± .25	0.002
Classical monocytes (%)	76.79 ± 3.28	84.12 ± 2.27	0.001
Non-classical monocytes (%)	12.55 ± 2.64	8.23 ± 1.71	0.001
Intermediate monocytes (%)	9.60 ± 0.94	7.03 ± 1.59	0.001

Independent sample T test. Data are represented as mean ± SD, $p < 0.05$ is significant.

BM and enter the peripheral blood and constitute about 10% of the total leucocyte count.[14] In the present study, the patients had a significant decrease in the absolute number of total monocytes (monocytopenia) when compared to the controls ($p = 0.002$). Our findings in pediatric patients with GD1 contribute to the etiopathogenesis of the immune abnormalities seen in GD patients. Previous studies on adults agreed with our data.[19,20] Bettman et al.[19] reported a significant reduction in the absolute number of total monocytes in patients when compared to the controls ($p < 0.05$). Mucci et al.[20] reported the same findings, with significantly lower total monocytes in 24 adult GD patients under ERT when compared to healthy adults. We detected a significant decrease in the frequencies of classical monocytes in the patients when compared to the controls ($p = 0.001$). Previous studies have reported the same findings.[19,20] Bettman et al.[19] found monocytopenia associated with significantly impaired monocyte migration capacity toward stromal-derived factor-1 alpha (SDF-1α). This defect may be due to the reduction in the expression of the surface SDF1α receptor and disturbed receptor–ligand binding in addition to augmented scavenging SDF-1α by C-X-C chemokine motif receptor 7 (seven transmembrane-spanning receptor).[19]

The reduction in monocyte counts in our patients and in the previous reports[19,20] could be explained by the decreased monocyte production caused by BM infiltration by Gaucher cells and the inhibition of monocyte growth due to accumulation of Gaucher cells.[4,21] Circulatory monocytes in GD displayed a significant suppression of superoxide generation and reduced potential for phagocytosis and staphylococcal killing.[22] Moreover, the upregulation of CD1d and MHC II molecules in GD monocytes cause increased activation of $CD4^{+}$ T lymphocytes.[23] ERT restore some of these monocyte functional defects and $CD4^{+}$ T cells numbers in the peripheral blood.[22]

On the other hand, we found significantly increased frequencies of $CD14^{-}$/dim$CD16^{++}$ (non-classical) monocyte subsets in the patients compared to the controls (p = 0.001). Previous

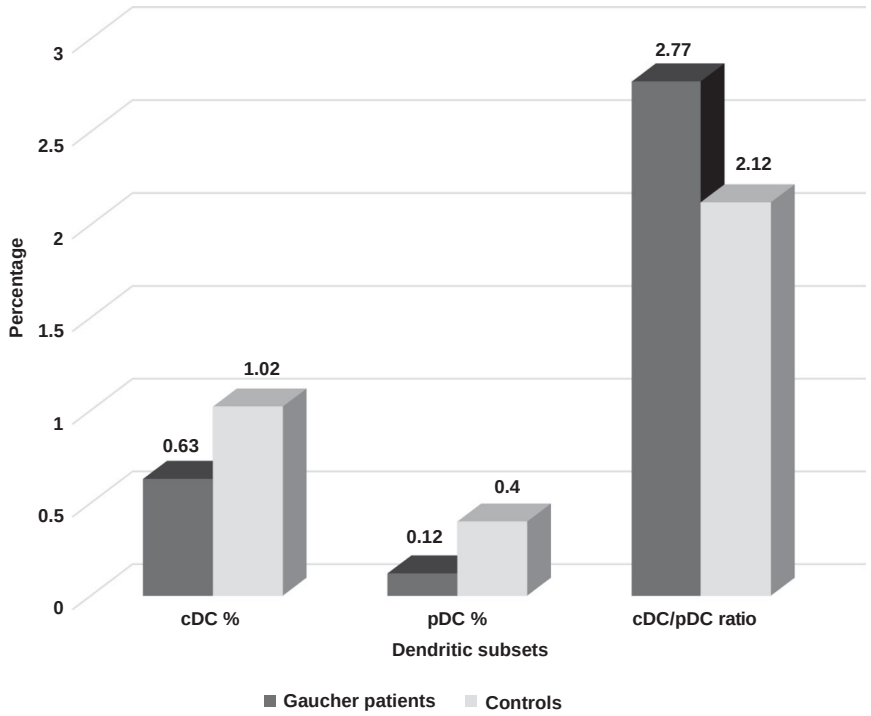

Fig. 3 Dendritic cell subsets in patients with Gaucher disease and controls.

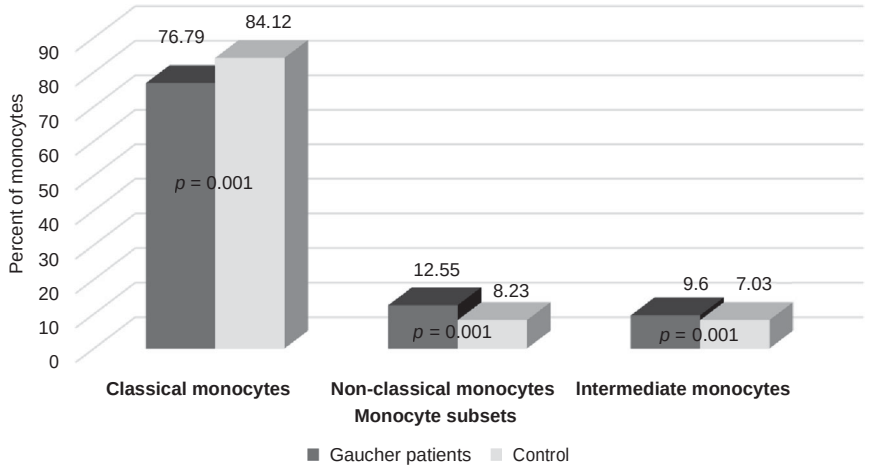

Fig. 4 Monocyte subsets in patients with Gaucher disease and controls.

studies[19,20,24] have shown a significant elevation of non-classical monocytes in adult GD patients as compared to healthy controls. In the present study, we observed for the first time a significantly higher frequency of intermediate monocytes ($CD14^+CD16^+$) in pediatric patients with GD1 as compared to healthy children (9.60 ± 0.94% versus 7.03 ± 1.59% controls, $p = 0.001$). Together with the previously reported information, our results propose a role for intermediate and non-classical monocytes in GD. These changes may contribute to the immune dysfunction state in GD1 children.

As this study is mainly descriptive, further studies are needed to determine the functional changes of DCs and monocytes and their immune dysfunction mechanism in pediatric patients with GD1.

CONCLUSION

Our results so far indicate that, compared to the control group, the GD1 children had significantly reduced total and classical monocyte, with significantly decreased frequencies for both types of DCs. These changes can contribute to immunological abnormalities in pediatric patients with GD1.

AUTHOR CONTRIBUTIONS

A.M.Z., K.S., A.-E.M.A., E.F.G., Y.F.A.-R., K.I.E., and K.H.M. designed the study, followed the patients, analyzed the data, and drafted the manuscript. Z.A.M.Z., A.M.Z., and E.M. N.A. performed all laboratory investigations of the study. A.E., K.S., and T.A. drafted the manuscript. All authors were involved in the critical analysis of the final version of the manuscript. All authors approved the manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Ethical approval: All protocols and investigations of our study followed the regulations of the research ethics committee of Assiut University (No. 120-2015).

Informed consentInformed consent was obtained from all guardians of children included in the study.

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