Immunological profiles of natural killer cells and their subpopulations in overweight and obesity class-I individuals stratified by two-dimensional categories of lipid profiles and body mass index

ARPA SURAPAITOON[1], KIATICHAI FAKSRI[1], PUNNAPAT NAWAWISHKARUN[1], SUPRANEE PHANTHANAWIBOON[1], KINGKAN JAISIRI[1], AUTTAWIT SIRICHOAT[1], PARAMA BUDMALA[1], ARNONE NITHICHANON[1,2], AMONRAT JUMNAINSONG[2,3], KANIN SALAO[4] and WISITSAK PHOKSAWAT[1,2]

[1]Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand; [2]The Centre for Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand; [3]Department of Clinical Immunology and Transfusion Sciences, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand; [4]Translational Oncology Research Center, Department of Biomedical Sciences, Vrije Universiteit Brussel, Brussels 1090, Belgium

Received December 4, 2025; Accepted August 7, 2026

DOI: 10.3892/br.2026.2192

Abstract. Overweight and obesity remain global health issues that should not be overlooked. They may impair immune health and alter the function of natural killer (NK) cells, immune effectors that may help regulate immune surveillance and associate metabolic inflammation. Therefore, the present case-control study investigated the immunological profiles of NK cells, both overall and by subpopulations [neural cell adhesion molecule 1 (NCAM1/CD56) staining: Weak ($CD56^{Dim}$) and strong ($CD56^{Bright}$)], in 125 Thai participants stratified into five groups by lipid profiles [normal lipid (NL) and dyslipidemia (DL) and body mass index (BMI; normal weight [NW], overweight [OW] and obesity class-I (OB-I)]. NK cells' immunological profiles were determined by flow cytometry. It was found that total NK cell percentages were significantly higher in the DL/OB-I group than in the NL/NW group ($P<0.01$) and the NL/OW group ($P<0.05$). Interferon-gamma (IFN-γ)-producing $CD56^{Bright}$ NK cell percentages were also highest in the DL/OB-I group. BMI correlated positively with total NK cell percentage ($R=0.214$; $P<0.05$), $CD56^{Dim}$ NK cell percentage ($R=0.217$, $P<0.05$), and IFN-γ-producing $CD56^{Bright}$ NK cell percentage ($R=0.463$; $P<0.01$). Altogether, the findings demonstrated that NK cell percentage and activation increased markedly with BMI and dyslipidemia. The immunological profiles of NK cells were particularly altered in the DL/OB-I group, characterized by higher percentages and cytokine-producing capacity. Therefore, BMI and lipid profiles must be controlled to preserve immunological status and prevent subsequent low-grade inflammation.

Correspondence to: Dr Wisitsak Phoksawat, Department of Microbiology, Faculty of Medicine, Khon Kaen University, 2 Mittraphap Road, Khon Kaen 40002, Thailand
E-mail: wisiph@kku.ac.th

Key words: NK cells, dyslipidemia, body mass index, obesity class-I, low-grade inflammation

Introduction

Overweight and obesity remain major global public health concerns, with prevalence and incidence rates continuing to rise worldwide (1,2). According to the World Health Organization (WHO), over 2.5 billion adults aged 18 years or older were overweight in 2022, of which over 890 million were classified as obese (www.who.int). Recently, the WHO updated BMI criteria for overweight and obesity class-I and class-II in adults in the Asia-Pacific region to 23.0-24.9, 25.0-29.9, and ≥30 kg/m^2, respectively (3,4). These revised cutoffs were proposed because Asians tend to be at greater risk of type 2 diabetes mellitus and cardiovascular diseases (CVDs) at lower BMIs than Caucasians (5).

Evidently, individuals who are overweight and obese are most at risk of dyslipidemia (6,7). Dyslipidemia and/or overweight/obesity are characterized not only by metabolic dysregulation but also by changes in innate and adaptive immune responses and, ultimately, higher chronic low-grade inflammation (8,9). NK cells are a key component of the innate immune system and may be altered under these aberrant conditions (10). As is well known, NK cells play vital roles in immunosurveillance and the elimination of virus-infected and malignant cells through signaling pathways that activate perforin-granzyme exocytosis, antibody-dependent cellular cytotoxicity and death receptor signaling mechanisms (11-13). Moreover, NK cells regulate immune homeostasis by secreting numerous cytokines and interacting with other immune cells to modulate inflammation (14). Typically, NK cells are divided

into two subpopulations, classified by the intensity of anti-CD56 (NCAM1) staining: Weak ($CD56^{Dim}$) and strong ($CD56^{Bright}$). $CD56^{Dim}$ NK cells mainly support cytotoxic activity by secreting perforin and granzymes, whereas $CD56^{Bright}$ NK cells function mainly as specialized immunoregulators and cytokine producers rather than primary killers (11,15,16).

Accumulating evidence suggests that metabolic abnormalities associated with excessive adiposity and/or dyslipidemia may drive quantitative, phenotypic and functional changes in NK cells. These changes may contribute gradually to impaired immune surveillance, increased susceptibility to viral infections and the development of non-communicable diseases (NCDs) (10,17-19). However, the underlying mechanisms and consequences of dyslipidemia- and/or excessive adiposity-induced alterations in NK cells remain poorly understood, especially in Asian individuals. Moreover, the reported data have often been conflicting, and definitive conclusions remain unclear.

Therefore, the present study examined the frequency and functional changes linking metabolic dysregulation and NK cells. It examines the immunological profiles of NK cells, both overall and by CD56-based subpopulations, in 125 Thai individuals stratified by lipid profiles and BMI. The findings are expected to be beneficial for preventive medicine, particularly in the context of NCDs.

Materials and methods

Study setting and participants. The present study was approved by the Ethical Committee of Khon Kaen University (approval number HE 671043) and was conducted in accordance with the 1964 Declaration of Helsinki. It examined leftover peripheral blood samples collected in ethylenediaminetetraacetic acid (EDTA) collection tubes from 125 Thai individuals aged 18-65 years (female/male, 68/57), obtained from the Medical Check-up Unit at Srinagarind Hospital (affiliated with Khon Kaen University) in Khon Kaen, Thailand (between February 2024 and June 2026). Individuals with underlying diseases, including hypertension, diabetes mellitus, immunological diseases, infectious diseases, and hematological diseases, were excluded, as were those taking anti-inflammatory drugs.

All participants were categorized into five subgroups based on their lipid profiles and BMI (classified according to the WHO criteria for Asians): Normal lipid and normal weight (NL/NW; BMI=18.5-22.9 kg/m^2), normal lipid and overweight (NL/OW; BMI=23.0-24.9 kg/m^2), dyslipidemia and normal weight (DL/NW; BMI=18.5-22.9 kg/m^2), dyslipidemia and overweight (DL/OW; BMI=23.0-24.9 kg/m^2) and dyslipidemia and obesity class-I (DL/OB-I; BMI=25.0-29.9 kg/m^2). DL was defined as at least one abnormal lipid profiles value according to the Japan Atherosclerosis Society's guidelines (20): Total cholesterol (TC) ≥200 mg/dl, triglyceride (TG) ≥150 mg/dl, low-density lipoprotein-cholesterol (LDL-C) ≥120 mg/dl and/or high-density lipoprotein-cholesterol (HDL-C) <40 mg/dl. Moreover, other laboratory parameters including fasting blood sugar (FBS), glycated hemoglobin A1c (HbA1c), creatinine (Cr), alkaline phosphatase (ALT) was also examined. The summarized data were represented in Table I.

Surface staining of NK cells. First, 100 μl of fresh peripheral whole blood (n=25 per group) was stained with the following monoclonal antibodies (mAbs) at dilution 1:50 in the dark for 10 min: Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (cat. no. 555916, BD Pharmingen; BD Biosciences) and peridinin-chlorophyll-protein complex-cyanine 5.5 (PerCP-Cy5.5)-conjugated anti-CD56 (cat. no. 318322, BioLegend, Inc.) at room temperature (RT) for 10 min. Next, red blood cells were lysed using FACS lysing solution (BD Biosciences) in the dark for 10 min. Then, the immune cells were washed with 1X phosphate-buffered saline (PBS) and then measured using a FACSLyric flow cytometer (BD Biosciences). Cells were categorized as NK cells if they were CD3-CD56+. NK cells were further stratified by CD56 staining intensity (15,16): weak ($CD56^{Dim}$) and strong ($CD56^{Bright}$). The percentages of NK cells were calculated relative to the total lymphocytes (FlowJo Software, v10; BD Biosciences). A representative example of NK cell detection, stratification, and analysis is shown in Fig. 1.

Intracellular staining to detect IFN-γ. Peripheral blood mononuclear cells (PBMCs) were isolated from six randomly selected samples per group using Lymphosep Lymphocyte Separation Medium (Biowest). Next, the cells were stimulated with phorbol-12-myristate-13-acetate (PMA; MilliporeSigma) and ionomycin calcium salt (MilliporeSigma) at 37˚C for 2 h at final concentrations of 100 ng/ml and 1,000 ng/ml, respectively, along with brefeldin A (Golgi apparatus inhibitor; eBioscience; Thermo Fisher Scientific, Inc.) at a final concentration of 3 μg/ml. Then, the cells were stained with FITC-conjugated anti-CD3 and PerCP-Cy5.5-conjugated anti-CD56 mAbs (dilution 1:50) in the dark at RT for 10 min, fixed with 2% formaldehyde at RT for 10 min, incubated with a permeabilization/wash buffer (R&D Systems, Inc.) at RT for 10 min, and stained with a Brilliant Violet 421 (BV421)-conjugated anti-IFN-γ mAb (cat. no. 502532, BioLegend, Inc.) at dilution 1:20, RT for 30 min. Finally, the cells were measured by FACSLyric flow cytometer (BD Biosciences), along with FACSuite software (v1.5, BD Biosciences). An unstimulated cell (medium) was measured in parallel. The percentages of all IFN-γ-producing NK cells and their subpopulations were referenced to the percentages within its own populations of total NK cells and each subset.

Statistical analysis. Data were analyzed by using FlowJo Software (BD Biosciences), all graphs were created using GraphPad Prism (Dotmatics) and all statistical analyses were performed using SPSS (v28, IBM Corp.). Data were compared between multiple groups using the Jonckheere-Terpstra test which was highly suitable multiple group analysis for trend data, followed by pairwise Dunn's tests if significant. Correlations between variables were assessed using Spearman's rank correlation coefficient (R). The effects of variable factors (sex, smoking and alcohol drinking as shown in Table I) on multiple outcome variables (the frequencies of total NK cells, their subpopulations, and their IFN-γ-producing counterparts, as displayed in Figs. 2 and 3) were assessed using multivariate analysis of covariance (MANCOVA) while controlling for covariates (age, blood pressures, pulse rate, and laboratory parameters as shown in Table I). All graphs were represented

Table I. Demographic and clinical characteristics of participants.

Parameter	Reference range	NL/NW (Mean ± SD)	NL/OW (Mean ± SD)	DL/NW (Mean ± SD)	DL/OW (Mean ± SD)	DL/OB-I (Mean ± SD)	P-values (Jonckheere-Terptra)
Number of subjects (n=125)		25	25	25	25	25	
Age (years)		46.4±7.7	47.0±10.2	48.3±9.8	49.1±10.3	47.5±9.6	NS
Sex (female/male)		16/9	10/15	14/11	14/11	14/11	NS
BMI (kg/m^2)		21.1±1.3	24.0±0.6[a]	20.8±1.4[b]	24.0±0.7[a,c]	26.5±1.3[a-d]	<0.001
Blood pressure							
Systolic (mmHg)	100-139	111.6±9.2	119.4±9.5[a]	119.8±8.6[a]	119.0±10.2[a]	124.3±10.6[a]	<0.001
Diastolic (mmHg)	60-89	66.7±11.4	71.2±8.4	69.6±8.4	71.8±8.7	76.1±7.7[a,c]	<0.01
PR (times/min)	60-100	72.5±9.8	72.6±9.9	71.5±9.0	74.8±10.5	72.0±10.1	NS
Blood sugar							
FBS (mg/dl)	70-110	88.8±9.3	88.8±9.2	88.9±10.7	89.1±6.4	90.7±6.7	NS
HbA1c (%)	4.6-7.0	5.3±0.4	5.4±0.6	5.6±0.3[a,b]	5.6±0.3[a,b]	5.6±0.4[a]	<0.01
Lipid profiles							
TC (mg/dl)	<200	171.6±18.6	173.9±16.5	219.3±29.8[a,b]	239.6±41.7[a,b]	231.2±36.8[a,b]	<0.001
HDL-C (mg/dl)	≥40	66.0±13.2	59.8±13.3	62.4±18.6	60.2±19.3	55.2±14.7[a]	<0.05
LDL-C (mg/dl)	<120	95.6±17.7	102.6±13.3	146.8±25.7[a,b]	162.2±40.1[a,b]	154.1±38.5[a,b]	<0.001
TG (mg/dl)	<150	73.3±32.6	86.6±34.0	103.0±36.0[a]	132.8±85.9[a]	161.4±119.2[a,b]	<0.001
Cr (mg/dl)	0.5-1.5	0.83±0.14	0.87±0.20	0.88±0.13	0.88±0.19	0.91±0.19	NS
ALT (U/l)	4-36	16.6±5.5	16.9±8.7	26.2±17.1[a,b]	22.4±11.1	25.0±15.0[a,b]	<0.05
Behavior factors							
Smoking		2	2	2	0	1	NS
Alcohol drinking		3	1	5	3	3	NS

[a-c] and [d] significant (P<0.05) pairwise differences (post hoc Dunn's tests) compared with the NL/NM, NL/OW, DL/NW, or DL/OW group, respectively. ALT, alkaline phosphatase; BMI, body mass index; Cr, creatinine; DL, dyslipidemia; FBS, fasting blood sugar; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; n, number of samples; NL, normal lipid; NS, not significant OB-I, obesity class I; OW, overweight; PR, pulse rate; SD, standard deviation; TC, total cholesterol; TG, triglyceride; NS, no significance.

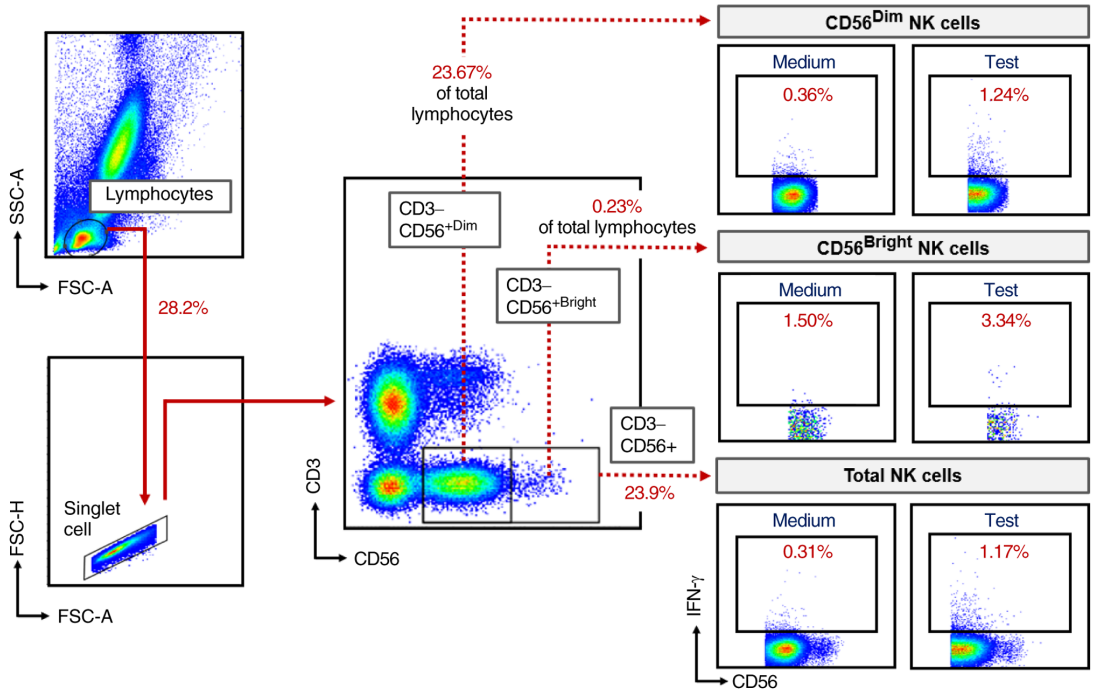

Figure 1. Representative example of NK cell detection, stratification into subpopulations ($CD56^{Dim}$ and $CD56^{Bright}$ NK) and analysis by flow cytometry. Lymphocytes were first gated into singlet cell prior to NK cells (CD3-CD56+), which were then gated into $CD56^{Dim}$ and $CD56^{Bright}$ NK subpopulations based on CD56 staining intensity. IFN-γ-producing NK cells were examined separately among total NK cells and each subpopulation by comparing unstimulated (medium) and stimulated (test) conditions. NK, natural killer; IFN-γ, interferon-gamma; FSC-A, forward scatter area; FSC-H, forward scatter height.

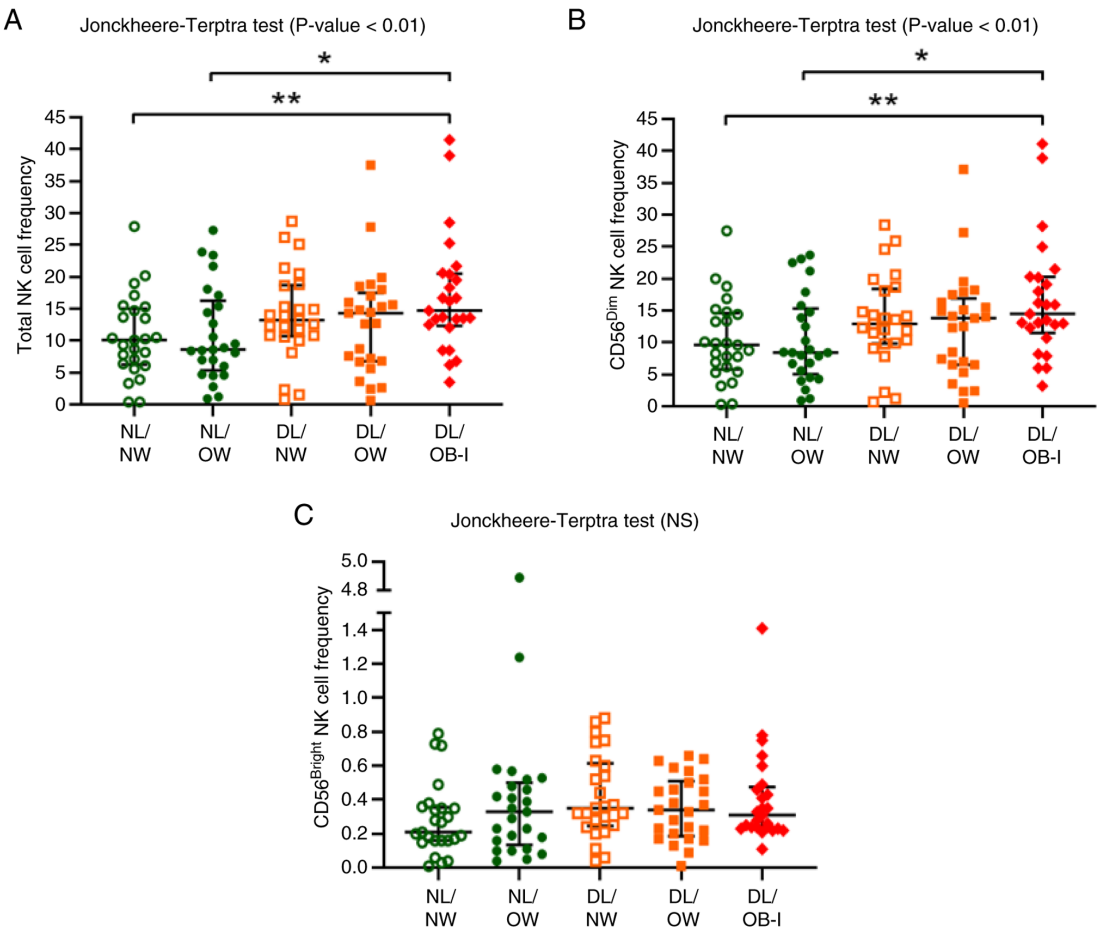

Figure 2. Percentages of total NK cells, $CD56^{Dim}$ and $CD56^{Bright}$ NK subpopulations. Scatter dot plots showing the percentages of (A) total NK cells, (B) $CD56^{Dim}$ NK cells, and (C) $CD56^{Bright}$ NK cells in each participant, stratified by group. The horizontal line indicates the median, and the vertical line denotes the IQR. $^{*}P<0.05$; $^{**}P<0.01$. NK, natural killer; IQR, interquartile range; NS, no significance.

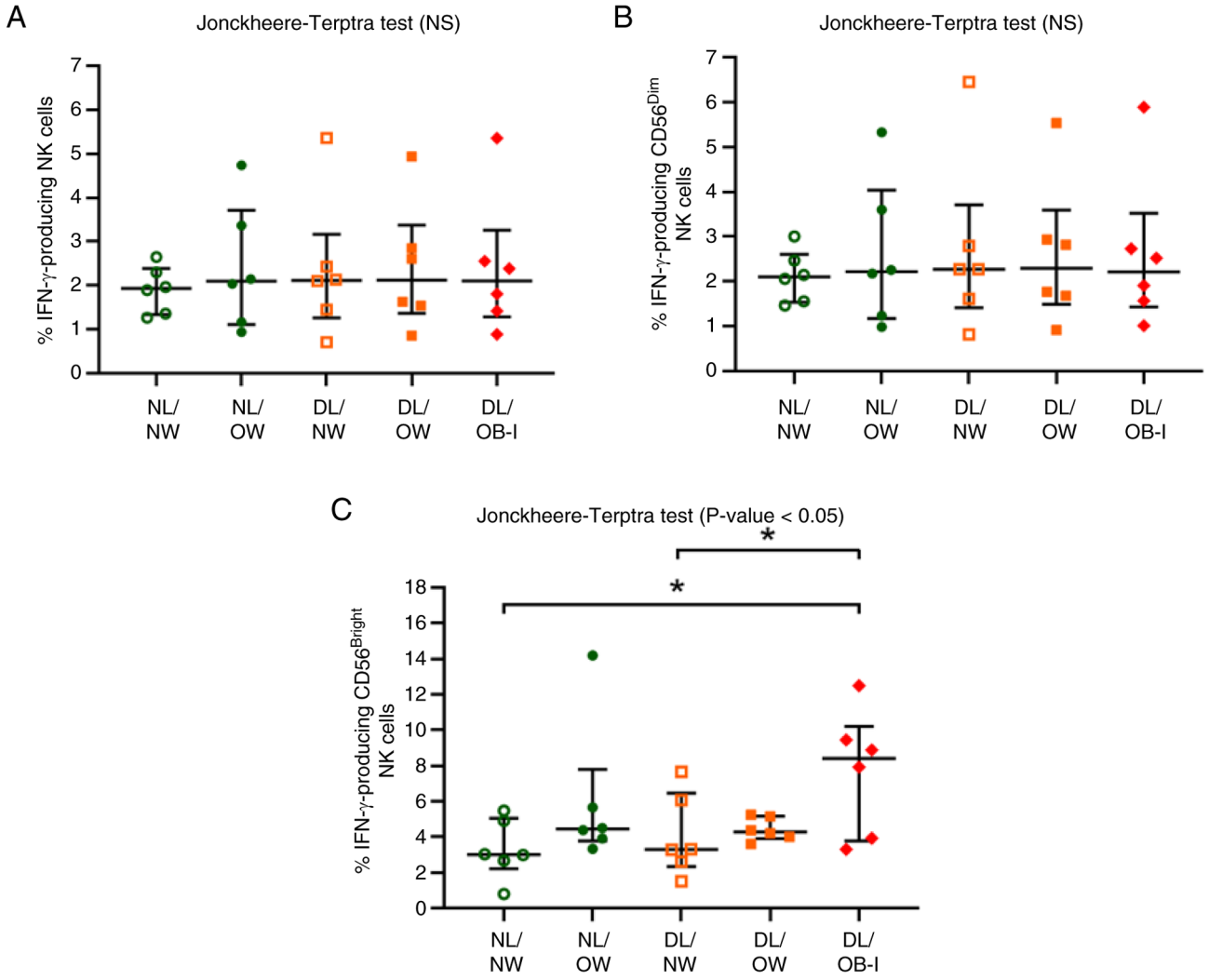

Figure 3. Percentages of IFN-γ-producing total NK cells, $CD56^{Dim}$ and $CD56^{Bright}$ NK subpopulations. Scatter dot plots showing the percentage of (A) IFN-γ-producing total NK cells, (B) IFN-γ-producing $CD56^{Dim}$ NK cells, and (C) IFN-γ-producing $CD56^{Bright}$ NK cells in each group. PBMCs isolated from six participants per group were examined. The horizontal line indicates the median, and the vertical lines denote the IQR. *P<0.05. IFN-γ, interferon-gamma; NK, natural killer; PBMCs, peripheral blood mononuclear cells; IQR, interquartile range; NS, no significance.

as medians of percentages with the inter quartile range (IQR). P<0.05 was considered to indicate a statistically significant difference.

Results

Participants' demographic and clinical characteristics. The present study enrolled leftover samples obtained from a total of 125 Thai individuals aged 18-65 years. Since the dataset had a trending pattern, the Jonckheere-Terpstra test was used to compare multiple groups. As well as BMI and lipid profiles, systolic and diastolic blood pressures, HbA1c and ALT were also statistically significant. However, the present study did not find any overall differences in age, sex, pulse rate (PR), FBS, Cr, or behavioral factors (including smoking and alcohol drinking). The pairwise comparisons for all variables are also shown in Table I.

Total and subpopulation percentages of NK cells by lipid profiles and BMI category. The present study analyzed the frequencies of total NK cells and NK subpopulations from five subgroups of participants. For multiple group analysis using the Jonckheere-Terpstra test, total NK cells had a statistical significance (P<0.01). For pairwise Dunn's tests, although total NK cell percentages were higher in all DL groups, they were only significantly higher in the DL/OB-I group than in the NL/NW (P<0.01) and NL/OW (P<0.05) groups (Fig. 2A). Regarding NK cell subpopulations, multiple analyses exhibited statistically significant for $CD56^{Dim}$ NK cells (P<0.01, Fig. 2B) but not for $CD56^{Bright}$ NK cells (Fig. 2C). In pairwise comparisons, $CD56^{Dim}$ NK cell percentages were highest in the DL/OB-I group, differing significantly from those in the NL/NW (P<0.01) and NL/OW (P<0.05) groups. A summary of the statistical values of total NK cell and subpopulation frequencies is shown in the Table SI.

IFN-γ-producing total NK cells and their subpopulations. Given that NK cells are the primary producers of IFN-γ, which determines their effector functions, differences in IFN-γ production capacity of NK cells by lipid profiles and/or BMI were examined, both overall and by subpopulations. Multiple group analysis represented that IFN-γ-producing total NK

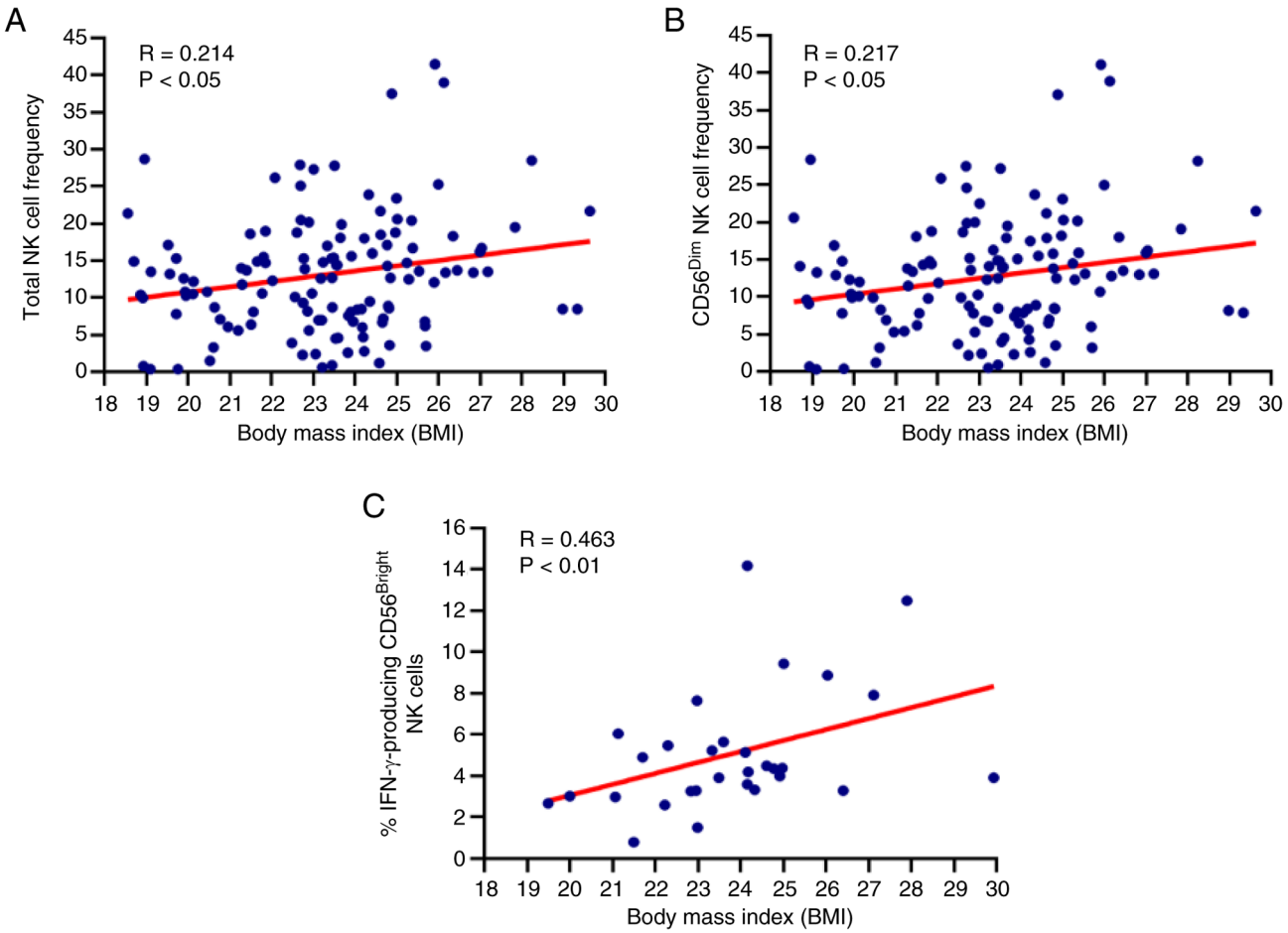

Figure 4. Correlations between BMI and percentages of total NK cells, $CD56^{Dim}$ NK and IFN-γ-producing $CD56^{Bright}$ NK subpopulations. Scatter dot plots showing correlations between BMI and the percentages of (A) total NK cells, (B) $CD56^{Dim}$ NK cells, and (C) IFN-γ-producing $CD56^{Bright}$ NK cells. The red line denotes the trend line. BMI, body mass index; NK, natural killer; PBMCs, peripheral blood mononuclear cells; NS, no significance.

cells and $CD56^{Dim}$ NK cells had no statistical significance (Fig. 3A and B). However, IFN-γ-producing $CD56^{Bright}$ NK cells exhibited statistically significance at $P<0.05$. In addition to pairwise analysis, the percentages of IFN-γ-producing $CD56^{Bright}$ NK cells was highest in the DL/OB-I group, differing significantly from those in the NL/NW and DL/NW groups ($P<0.05$, Fig. 3C). The summary of the statistical values of IFN-γ-producing NK cell and their subpopulation percentages across five subgroups is additionally provided in Table SII.

Correlations between BMI and NK cell percentages, and IFN-γ production. Finally, correlations between BMI and NK cell percentages were examined, both overall and by subpopulations, and IFN-γ production status. BMI correlated positively with total NK cell percentages ($R=0.214$, $P<0.05$; Fig. 4A), $CD56^{Dim}$ NK cell percentages ($R=0.217$, $P<0.05$; Fig. 4B), and IFN-γ-producing $CD56^{Bright}$ NK cell percentages ($R=0.463$, $P<0.01$; Fig. 4C).

Discussion

Overweight and obesity remain major health problems in the era of globalization, and their incidences are likely to increase in the future (1,2). Overweight and obesity are associated with a number of modifiable and non-modifiable risk factors, including lifestyle, low physical activity, diet, advanced age, genetics and the environment (21). Numerous studies on obesity/dyslipidemia have shown alterations in immune cell populations, phenotypes, and functions, predominantly T, NKT-like, and NK cells (10,17,22-24). However, research on NK cell changes remains limited, and the evidence remains inconsistent regarding NK cells' total and subpopulation percentages and functional capacities (10). Moreover, representative data on individuals with a BMI of 25.0-29.9 (obesity class-I) and their lipid profiles remain limited.

Therefore, the present study was the first, to the best of the authors' knowledge, to compare NK cell percentages, both overall and by subpopulations, and IFN-γ production status among 125 Thai (Southeast Asian) individuals without underlying diseases or anti-inflammatory using stratified by two-dimensional categories: Lipid profiles (normal lipid and dyslipidemia) and BMI (normal weight, overweight and obesity class-I). The participants were therefore distinguished into five subgroups (Table I). This classification differs from that used in prior studies, which mainly compared normal lipid vs. dyslipidemia or normal BMI vs. class-II obesity (BMI ≥30.0 kg/m^2). Therefore, the present study provided a finer-grained analysis of NK cell changes associated with metabolic alterations and increasing adiposity, which may help

reveal such changes. However, individuals with normal lipid plus obesity class-I were not included in the present study due to their rarity in the Thai population.

NK cells were examined, both overall and by subpopulations, by flow cytometry and compared across the five subgroups. Total NK cell percentages were higher in the DL groups and the group-wise comparisons exhibited a corresponding stepwise escalation. However, they were only markedly higher in the DL/OB-I group compared with the other groups. It was hypothesized that dyslipidemia may associate with the expansion of the total NK cell population, especially in obesity class-I. For instance, Wouters, *et al* (18) found that subjects with obesity had markedly higher total NK cell percentages than those who were lean. Obese individuals may associate greater low-grade inflammation. By contrast, Xu *et al* (22) found higher NK cell percentages in Chinese adults with dyslipidemia than in healthy controls, although the difference was not significant. Bähr *et al* (24) found no significant difference between subjects with obesity (BMI >30.0 kg/m^2) and normal weight (BMI <25.0 kg/m^2).

Regarding NK cell subpopulations, although CD56^{Dim} NK cell percentages were higher in the DL groups, only those in the DL/OB-I group were markedly higher than those in the other groups. This finding contrasted with a previous study on obesity (24). However, it was therefore hypothesized that under dyslipidemia, especially in higher BMI, individuals may experience greater low-grade inflammation. For CD56^{Bright} NK subpopulation, multiple group analysis showed no statistical significance.

Phan *et al* (25) previously showed that NK cell populations differ markedly by sex, both overall and by subpopulation. Thus, the present study further investigated whether sex affects NK cell populations using data from the NL/NW group, as it should best reflect sex differences without the confounding effects of dyslipidemia and high BMI. The proportions of total NK cells and CD56^{Dim} NK cells were significantly higher in males than in females (P<0.05; Fig. SI), consistent with Phan *et al* (25). However, the proportion of CD56^{Bright} NK cells did not differ markedly across groups. Nevertheless, the group-to-group comparison is unlikely to have been affected, as the sex ratio was adjusted to be comparable across subgroups.

The present study then observed the percentages of IFN-γ-producing total NK cells and their subpopulations. IFN-γ-producing CD56^{Bright} NK cell percentages were markedly higher in the DL/OB-I group than in the NL/NW and DL/NW groups. Accordingly, it is hypothesized that higher BMI may be associated with stronger immunological response driven by greater cytokine-induced low-grade inflammation. Consistent with a previous study (24), IFN-γ-producing CD56^{Bright} NK cell percentages were markedly higher in obese individuals who had BMI ≥30.0 than in normal weight groups (BMI <25.0), whereas IFN-γ-producing CD56^{Dim} NK cell percentages were markedly lower. However, the present study was limited to functional cytokine production capacity based on cellular responses stimulated by PMA (protein kinase C activator) and calcium ionophore (ionomycin), which activate the phosphoinositide 3-kinase (PI3k)/Akt signaling pathway (26). The present study was primarily an observational one that paves the way for further investigations into other functional aspects. In feasible cases of dyslipidemia combined with obesity condition, higher pro-inflammatory adipokines such as leptin and visfatin, as well as excessive long-chain fatty acid, were associated with signaling pathways involved in IFN-γ production. Possibly, leptin may activate Janus kinase 2/signal transducer and activator of transcription 3 (STAT3) prior to stimulating STAT4, T-box expressed in T cells (T-bet) transcription factor and ultimately IFN-γ production (27,28). As well as visfatin, it probably induces IFN-γ production via nuclear factor kappa-light-chain-enhancer of activated B cells and STAT3 signaling pathways (29). Additionally, long-chain fatty acids can be obtained from dietary fats and triglyceride components and also promote this cytokine production. However, the associated pathway remains inconclusive (30).

In other functional considerations of interest, previous studies have investigated the effects of obesity on NK cytotoxicity-related assay and degranulation function, consistently showing decreased cytotoxic activity against tumor cells (31-34) and reduced degranulation, as indicated by decreased lysosome-associated membrane protein 1/CD107a expression (31,33) or granzyme B production (33). However, Zhang *et al* (19) demonstrated that NK cell cytotoxicity, measured at an effector-to-target cell (human leukemic cell line K562) ratio of 12.5:1, was significantly higher in women with recurrent pregnancy loss and dyslipidemia than in those with normal lipid levels (P<0.05).

Since the clinical characteristics of various participants were found to be statistically significant following the Jonckheere-Terpstra test, MANCOVA was also examined the effects of variable factors on multiple outcomes of all NK cell percentages and cytokine production capacity while controlling for covariates. However, significant associations were not found. In the present study, age, PR, FBS, Cr, as well as variables including sex, smoking, and alcohol drinking, were tested simultaneously.

BMI correlated positively with the percentages of total NK cells, CD56^{Dim} NK cells, and IFN-γ-producing CD56^{Bright} NK cells. Thus, BMI may be possible physical indicator reflecting immunological status for NK cell and their function. Nevertheless, the observed significant positive correlations were also largely driven by dyslipidemia, especially in the DL/OB-I group, which made the effect appear more pronounced. Similarly, Viel *et al* (17) found that NK cell counts in peripheral blood correlated positively with BMI. While the findings of Bähr *et al* (24) are largely consistent, the authors reported negative correlations between BMI and both CD56^{Dim} NK percentage and cytokine production. Nevertheless, the existing evidence is based on only a few studies and further studies are required to advance and validate this concept.

Overall, it appeared that the findings of the present study leaned toward a higher low-grade inflammatory outcome. Larger NK cell populations and changes in their function might help drive occurring of the development of NCDs such as CVDs, kidney diseases and type 2 diabetes mellitus. In CVDs, NK cells were predominantly increased in human atherosclerotic lesions (35,36). Consistently, symptomatic patients showed increased percentages of IFN-γ-producing NK cells and CD56^{Bright} NK cells in atherosclerotic plaques (37), as well as higher levels of circulating NK cells (38). Moreover, elevated numbers of IFN-γ-producing NK cells increase

cholesterol accumulation in macrophages, inducing foam cell formation and potentially activating macrophages, which, in turn, release matrix metalloproteinases to degrade endothelial cells, vascular smooth muscle cells and the extracellular matrix, ultimately leading to destabilization and rupture of atherosclerotic plaques (39,40). In kidney diseases, greater infiltration of activated NK cells might contribute to inflammation, stressed tubular and endothelial cells, interstitial fibrosis, and chronic damage to glomerular tissue through IFN-γ-mediated functions and cytolytic mediators (41-43). In non-diabetic individuals, a previous study showed that fasting glucose levels correlated positively with the number of total NK cells (44). An increase in NK cells may serve as a marker of immune activation and may also be associated with higher low-grade inflammation. However, the present study found no significant differences in FBS among the groups (all average values were within the normal range) and no correlation between FBS and NK cell total or subpopulation percentages, or between FBS and IFN-γ-producing NK cell total or subpopulation percentages. In type 2 diabetes mellitus, NK cells could contribute to insulin resistance primarily through obesity-induced low-grade inflammation by enhancing the release of proinflammatory mediators, such as the tumor necrosis factor-α and IFN-γ, which could disrupt the normal insulin signaling pathway and down-regulate key insulin signaling components, including the insulin receptor, insulin receptor substrate 1 and the insulin-regulated glucose transporter type 4 (45,46). Therefore, greater numbers of NK cells and IFN-γ-producing NK cells might contribute to, and enhance, the microenvironment associated with the pathogenesis of these NCDs.

In conclusion, the present study was the first, to the best of the authors' knowledge, to report the immunological profiles of NK cells, both overall and by subpopulations, in Thai individuals stratified by lipid profiles and BMI, thereby likely revealing clearer patterns than would be possible by assessing each variable alone. The immunological status of individuals with dyslipidemia was shifted toward a more proinflammatory state. Notably, those with dyslipidemia and obesity class-I exhibited predominant changes in both NK cell percentage and function. Therefore, both BMI and lipid profiles must be controlled to prevent immunological changes and potentially reduce low-grade inflammation. However, even although the sample size achieved a test power (1-β) >80%, the small number of participants remains a limitation of the present study.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Young Research Development Project of Khon Kaen University Year 2024, Khon Kaen University, Thailand.

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ASu was responsible for conceptualization, review, sample collection, data analysis, and writing of the first draft of the manuscript. PN was responsible for sample administration and data curation. ASu and PN confirm the authenticity of all the raw data. KF, SP, PB, ASi, AJ, AN, KJ and KS were responsible for conceptualization and manuscript review and editing. WP was responsible for funding acquisition, conceptualization, data analysis, manuscript editing, final manuscript preparation and correspondence. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee for Human Research of Khon Kaen University (approval no. HE 671043) and conducted in accordance with the 1964 Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Wisitsak Phoksawat ORCID ID: 0000-0002-8866-9459

References

1. Islam ANMS, Sultana H, Nazmul Hassan Refat M, Farhana Z, Abdulbasah Kamil A and Meshbahur Rahman M: The global burden of overweight-obesity and its association with economic status, benefiting from STEPs survey of WHO member states: A meta-analysis. Prev Med Rep 46:102882, 2024.
2. Jeemon P and Sivasubramonian S: The silent surge: Obesity driving a global cardiovascular crisis. Glob Heart 20: 77, 2025.
3. World Health Organization. Regional Office for the Western Pacific. The Asia-Pacific perspective: Redefining obesity and its treatment. Sydney: Health Communications Australia, 2000.
4. Okawa Y, Mitsuhashi T and Tsuda T: The Asia-Pacific body mass index classification and new-onset chronic kidney disease in non-diabetic Japanese adults: A community-based longitudinal study from 1998 to 2023. Biomedicines 13: 373, 2025.
5. WHO Expert Consultation: Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 363: 157-163, 2004.
6. Nussbaumerova B and Rosolova H: Obesity and dyslipidemia. Curr Atheroscler Rep 25: 947-955, 2023.
7. Yudin R, Aman A, Rasyid H, Bakri S, Sanusi H, Daud N and Zainuddin A: Risk of dyslipidemia in obese young adult subjects as measured by various obesity indices. J Endocrinol Metab 12: 102-106, 2022.
8. Barakat B and Almeida MEF: Biochemical and immunological changes in obesity. Arch Biochem Biophys 708: 108951, 2021.
9. Shaikh SR, Beck MA, Alwarawrah Y and MacIver NJ: Emerging mechanisms of obesity-associated immune dysfunction. Nat Rev Endocrinol 20: 136-148, 2024.
10. Bähr I, Spielmann J, Quandt D and Kielstein H: Obesity-associated alterations of natural killer cells and immunosurveillance of cancer. Front Immunol 11: 245, 2020.
11. Mace EM: Human natural killer cells: Form, function, and development. J Allergy Clin Immunol 151: 371-385, 2023.

12. Zuo W and Zhao X: Natural killer cells play an important role in virus infection control: Antiviral mechanism, subset expansion and clinical application. Clin Immunol 227: 108727, 2021.
13. Sharma P, Kumar P and Sharma R: Natural killer cells-their role in tumour immunosurveillance. J Clin Diagn Res 11: BE01-BE05, 2017.
14. Zitti B and Bryceson YT: Natural killer cells in inflammation and autoimmunity. Cytokine Growth Factor Rev 42: 37-46, 2018.
15. Soelistyoningsih D, Susianti H, Kalim H and Handono K: The phenotype of CD3-CD56^{bright} and CD3-CD56^{dim} natural killer cells in systemic lupus erythematosus patients and its relation to disease activity. Reumatologia 60: 258-265, 2022.
16. Tahrali I, Kucuksezer UC, Akdeniz N, Altintas A, Uygunoglu U, Aktas-Cetin E and Deniz G: CD3$^-$CD56$^+$ NK cells display an inflammatory profile in RR-MS patients. Immunol Lett 216: 63-69, 2019.
17. Viel S, Besson L, Charrier E, Marçais A, Disse E, Bienvenu J, Walzer T and Dumontet C: Alteration of natural Killer cell phenotype and function in obese individuals. Clin Immunol 177: 12-17, 2017.
18. Wouters K, Gaens K, Bijnen M, Verboven K, Jocken J, Wetzels S, Wijnands E, Hansen D, van Greevenbroek M, Duijvestijn A, *et al*: Circulating classical monocytes are associated with CD11c$^+$ macrophages in human visceral adipose tissue. Sci Rep 7: 42665, 2017.
19. Zhang Y, Alzahrani M, Luu T, Fatunbi J, Ganieva U, Bilal M, Thees A, Fonseca Gonzalez M, Hawryluk D, Dambaeva S and Kwak-Kim J: Dyslipidemia is associated with increased natural killer cell cytotoxicity and autoimmunity in women with recurrent pregnancy losses. J Clin Endocrinol Metab 111: 229-243, 2025.
20. Teramoto T, Sasaki J, Ishibashi S, Birou S, Daida H, Dohi S, Egusa G, Hiro T, Hirobe K, Iida M, *et al*: Diagnostic criteria for dyslipidemia. J Atheroscler Thromb 20: 655-660, 2013.
21. Masood B and Moorthy M: Causes of obesity: A review. Clin Med (Lond) 23: 284-291, 2023.
22. Xu DM, Li Q, Yi JX, Cai XJ, Xie L, Fang W, Qiu JF, Xu CW, He CL, Xu XR, *et al*: Investigation of lymphocyte subsets in peripheral blood of patients with dyslipidemia. Int J Gen Med 14: 5573-5579, 2021.
23. Wang Y, Zou Y, Jiang Q, Li W, Chai X, Zhao T, Liu S, Yuan Z, Yu C and Wang T: Ox-LDL-induced CD80$^+$ macrophages expand pro-atherosclerotic NKT cells via CD1d in atherosclerotic mice and hyperlipidemic patients. Am J Physiol Cell Physiol 326: C1563-C1572, 2024.
24. Bähr I, Jahn J, Zipprich A, Pahlow I, Spielmann J and Kielstein H: Impaired natural killer cell subset phenotypes in human obesity. Immunol Res 66: 234-244, 2018.
25. Phan MT, Chun S, Kim SH, Ali AK, Lee SH, Kim S, Kim SH and Cho D: Natural killer cell subsets and receptor expression in peripheral blood mononuclear cells of a healthy Korean population: Reference range, influence of age and sex, and correlation between NK cell receptors and cytotoxicity. Hum Immunol 78: 103-112, 2017.
26. Gao R, Li X, Gao H, Zhao K, Liu X, Liu J, Wang Q, Zhu Y, Chen H, Xiang S, *et al*: Protein phosphatase 2A catalytic subunit β suppresses PMA/ionomycin-induced T-cell activation by negatively regulating PI3K/Akt signaling. FEBS J 289: 4518-4535, 2022.
27. Miao D and Zhang L: Leptin modulates the expression of catabolic genes in rat nucleus pulposus cells through the mitogen-activated protein kinase and Janus kinase 2/signal transducer and activator of transcription 3 pathways. Mol Med Rep 12: 1761-1768, 2015.
28. Tian H, Xing J, Tang X, Chi H, Sheng X and Zhan W: Identification and characterization of a master transcription factor of Th1 cells, T-bet, within flounder (Paralichthys olivaceus). Front Immunol 12: 704324, 2021.
29. Heo YJ, Choi SE, Jeon JY, Han SJ, Kim DJ, Kang Y, Lee KW and Kim HJ: Visfatin induces inflammation and insulin resistance via the NF-κB and STAT3 signaling pathways in hepatocytes. J Diabetes Res 2019: 4021623, 2019.
30. Pompura SL, Hafler DA and Dominguez-Villar M: Fatty acid metabolism and T cells in multiple sclerosis. Front Immunol 13: 869197, 2022.
31. Laue T, Wrann CD, Hoffmann-Castendiek B, Pietsch D, Hübner L and Kielstein H: Altered NK cell function in obese healthy humans. BMC Obes 2: 1, 2015.
32. O'Shea D, Cawood TJ, O'Farrelly C and Lynch L: Natural killer cells in obesity: Impaired function and increased susceptibility to the effects of cigarette smoke. PLoS One 5: e8660, 2010.
33. Mylod E, O'Connell F, Donlon NE, Davern M, Marion C, Butler C, Reynolds JV, Lysaght J and Conroy MJ: Real-time ex vivo monitoring of NK cell migration toward obesity-associated oesophageal adenocarcinoma following modulation of CX3CR1. Sci Rep 14: 4017, 2024.
34. Naujoks W, Quandt D, Hauffe A, Kielstein H, Bähr I and Spielmann J: Characterization of surface receptor expression and cytotoxicity of human NK cells and NK cell subsets in overweight and obese humans. Front Immunol 11: 573200, 2020.
35. Bobryshev YV and Lord RSA: Identification of natural killer cells in human atherosclerotic plaque. Atherosclerosis 180: 423-427, 2005.
36. Selathurai A, Deswaerte V, Kanellakis P, Tipping P, Toh BH, Bobik A and Kyaw T: Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. Cardiovasc Res 102: 128-137, 2014.
37. Bonaccorsi I, Spinelli D, Cantoni C, Barillà C, Pipitò N, De Pasquale C, Oliveri D, Cavaliere R, Carrega P, Benedetto F and Ferlazzo G: Symptomatic carotid atherosclerotic plaques are associated with increased infiltration of natural killer (NK) cells and higher serum levels of NK activating receptor ligands. Front Immunol 10: 1503, 2019.
38. Clerc G and Rouz PM: Lymphocyte subsets in severe atherosclerosis before revascularization. Ann Intern Med 126: 1004-1005, 1997.
39. Voloshyna I, Littlefield MJ and Reiss AB: Atherosclerosis and interferon-γ: New insights and therapeutic targets. Trends Cardiovasc Med 24: 45-51, 2014.
40. Dumitriu IE, Araguás ET, Baboonian C and Kaski JC: CD4$^+$ CD28 null T cells in coronary artery disease: When helpers become killers. Cardiovasc Res 81: 11-19, 2009.
41. Turner JE, Rickassel C, Healy H and Kassianos AJ: Natural killer cells in kidney health and disease. Front Immunol 10: 587, 2019.
42. Law BMP, Wilkinson R, Wang X, Kildey K, Lindner M, Rist MJ, Beagley K, Healy H and Kassianos AJ: Interferon-γ production by tubulointerstitial human CD56^{bright} natural killer cells contributes to renal fibrosis and chronic kidney disease progression. Kidney Int 92: 79-88, 2017.
43. Zhang ZX, Wang S, Huang X, Min WP, Sun H, Liu W, Garcia B and Jevnikar AM: NK cells induce apoptosis in tubular epithelial cells and contribute to renal ischemia-reperfusion injury. J Immunol 181: 7489-7498, 2008.
44. Schmitz T, Freuer D, Linseisen J and Meisinger C: Associations between blood markers of glucose metabolism and characteristics of circulating lymphocytes. Clin Nutr 43: 285-295, 2024.
45. Lee BC, Kim MS, Pae M, Yamamoto Y, Eberlé D, Shimada T, Kamei N, Park HS, Sasorith S, Woo JR, *et al*: Adipose natural killer cells regulate adipose tissue macrophages to promote insulin resistance in obesity. Cell Metab 23: 685-698, 2016.
46. Li Y, Wang F, Imani S, Tao L, Deng Y and Cai Y: Natural killer cells: Friend or foe in metabolic diseases? Front Immunol 12: 614429, 2021.