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Association between Natural Killer Cell Cytotoxicity and the Progression of Non-Small Cell Lung Cancer

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ABSTRACT

The present study aims to assess the role of NK-cell in the prognosis of NSCLC.

Samples and Methods: A total of sixty newly diagnosed patients with NSCLC were included in this study. Fifteen of them were female and (45) male and then patients were followed up for six months. The diagnosis is revised by the supervisor Oncologist. ELISA technique was used to investigate the two immunological variables (NK & INF-γ). The results show, the mean of NK cell significantly increased from as small as (8.016) among NSCLC cases with an early stage to as high as (14.293) among cases with advanced stages (P =0.001), while in INF-γ test,the quantity of INF-γ was decreased gradually in a mean of (0.13) among metastatic stages of pulmonary cancer compared with an early stage where mean about (0.229); P=0.001. Furthermore, the linear correlation coefficients show there isa strong positive correlation between NK cell very strong positive relationship between the level of NK cell (NKG2A/CD94) and staging (r= 0.608). Meanwhile, strong negative linear correlation coefficients that mean the level of INF-γ decrease with a bad prognosis of NSCLC patients (r= - 0.486). Moreover, by using Kaplan-Mier test the mortality rate of NSCLC patients increased (45% & 59%) with high levels of NK cells concentration respectively during a maximum of six months follow up periods. Meanwhile,the cumulative proportion of deaths in NSCLC patients at six months increased with the lowest tercile of INF-γ (60%). A high expressionof NKG2A/CD94 of $CD56^{bright}$ $CD16^{-}$ in peripheral blood of NSCLC patients correlates with bad prognosis which may be used as the prognostic marker while a high level of INF-γ refers to the good prognosis.Low cytotoxicity of NK correlated with a low level of INF-γ and both of them decreased in concentration after three and six months of follow up post- treatments.

Keywords: Natural killer, NKG2A/CD94, INF-γ, NSCLC

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INTRODUCTION

Lung cancer is one of the most common malignancies and the leading cause of cancer related mortality in both men and women around the world where it is the most malignant tumor in man and the second after the breast cancer in women (1). Approximately 80-85% of all lung cancer cases are non-small cell lung cancer (NSCLC) (2). In Iraq, lung cancer is the most commonly occurring cancer in men (14.76%-16.7%) and the fifth in women (4.2%) for the period of 1995-2011(3). Cigarette smoking is by far the most important single factor that causes lung cancer; it is directly responsible for 90% of lung carcinomas. Although, several modern techniques which helped early diagnosis of lung cancer, advance surgical resection and multimodal treatment, the mortality rate by lung cancer increases because of its recurrence in a so short period, that it has been more aggressive and fatal than the first form (4) that is because the microenvironment of tumor plays a critical role in eliminating and cleaning the body from transformed cell and immune cells as part of this environments help recognize and kill transformed cell in early stage by secretion of cytokines and recruits other immune cells (5). Therefore, there is a principle need to study natural immunity which mediated by NK cell which will facilitate the choice of the patients with a high chance of carcinoma. NK cells can play a critical role as a part of natural immunity against malignancy. They are effective at the first line defense for tumor elimination; their major functions include cytotoxicity and cytokine production that helps in killing oncogenic cells (6). NK cells mediated defense against NSCLC, the previous study shows this role through isolating of NK from tumor cells and also NK cell in tumor tissue appear in an inhibitory form compare with NK in peripheral blood or normal pulmonary tissue (7). The NK cells are forming about (5-10%) from the total cells in circulation and can vary in number and activity at different ages (8). It can respond to activation via several chemokine and interleukins and after being activated it migrate, to the site of infection and secreted perforin, granzyme, and INF-γ; its activation occurs without the requirement for transcription or proliferation. NK cells responses are mediated through cell surface receptors that can either be inhibitory or activating (9). There are two main NK cell subsets, one of them is that of $CD56^{dim}$ $CD16^{+}$ and $CD56^{bright}CD16^{-}$(10–12). There is still limited data regarding the effects of NK cells on lung cancer prognosis. The present study investigates the role of serum $CD56^{bright}CD16^{-}$ expression in NSCLC patients crosstalk with innate immunity through NK cell (13) and the study also, focuses on the $CD56^{bright}CD16^{-}$ asubset of NK cell which has special molecules on the surface that refer to inhibitory receptors NKG2A and lacks KIRs (8).

MATERIALS & METHODS

Study Design

A longitudinal study was carried out in the Department of Microbiology / Faculty of Medicine / University of Kufa. A total of sixty newly diagnosed patients with NSCLC were included in this study, from Middle Euphrates Cancer Centre (MECC), in the period between April 2017 to May 2018 where (15) female and (45) male and then patients

followed up post treatment for six months. All cases are diagnosed by a physician according to NCCN (National Comprehensive Cancer Network) criteria, Staging was established according to the eighth edition of TNM classification for lung cancer(14).Study protocol approved by Faculty of Medicine / University of Kufa ethical committee.

Inclusion Criteria

- Histological type of NSCLC
- All age groups.
- Both genders.
- Social history like (smoking, alcohol, and drug-addicted) and family history for lung cancer this information taken from patients through a questionnaire prepared for interview previously.

Exclusion Criteria

The patients with any illness are all excluded from the study design by history.

Outcomes

- Serum level of NK cell CD56 ^{bright} CD16^{-}.
- Serum level of INF-γ.
- Chest X-Ray.
- CT-scan.

The Collected Samples

Blood samples were collected by vein puncture 5 milliliter (ml) of venous blood withdrawn from newly diagnosed NSCLC by using disposable syringes under aseptic technique; they then transferred to 10 ml sterile plain tube, centrifuge at 2500 rpm for 10 minutes and the separated serum was divided into several aliquots and immediately frozen at –20ºC till further use to avoid repeated thawing and freezing for human NKG2A/ CD94 NK CD56 ^{bright} CD16^{-}& INF-γ antigen test.by using human,NKG2A/CD94, and INF-γ quantitative ELISA kit are used in *vitro* to the determination of human NKG2A/CD94 and INF-γ in serum and this ELISA Kit is for research use only, the detection range of this kit about (0.31-20 ng/ml) for NKG2A/CD94 and about (12.5-400ng/ml) for INF-γ.

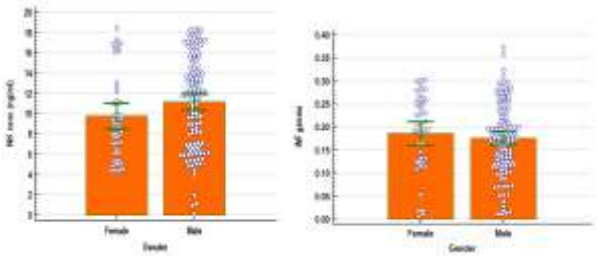
Statistical Analysis

Data were translated into a computerized database structure. An expert statistical advice was sought for. Statistical analyses were computer-assisted using SPSS version 23 (Statistical Package for Social Sciences). Frequency distribution for selected variables was done first. Horizontal bars indicate the means. For multiple comparisons, *p* values will be calculated by a one way ANOVA. Spearman correlation test was used to evaluate the association between NK levels, and INF-γ levels and the correlation between these three variables and tumorstaging. The Kaplan-Meier method with the log-rank test was used to calculate survival rates and differences in survival curves. A receiver operating characteristic (ROC), or simply ROC curve, is a graphical plot which illustrates the performance of a binary classifier system as its discrimination threshold is varied. It is created by plotting the fraction of true positives (sensitivity) out of the positives *versus* the fraction of false positives (specificity) out of the negatives, at various threshold settings. $P < 0.05$ was considered statistically significant.

RESULTS

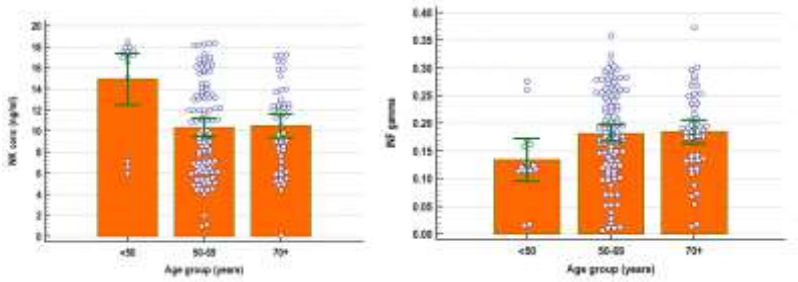
The Levels of NK&INF-γ according to a Demographic and Clinical Characteristic of Patients

The levels of NK (NKG2A/CD94) and INF-γ were not affected by gender; that means there was no statistically significant difference in mean between males and females (Figure 3-1). According to the age category, the present study included all age group, there are three age groups of (50 or less, 50-69 and 70 or more years old). In NK, the level of the inhibitory receptor (NKG2A/CD94) decrease in the age group 70 + where mean (10.536) is low compared with < 50 age group where mean is (14.965). There are statistical differences between age group and NK level (P<0.001). Where there is no statistical difference between the INF-γ levels and age group (Table3-2). Further, according to linear correlation coefficients, there are weak negative relationship (r= -0.256), while in concentration of INF-γ there are statistically differences where mean is (0.1854, 0.1522 &0.1348) P=0.12, in 70+, 50-90&< 50 age group respectively and coefficients was weak (r=0.198). In histopathological character, there was no significant difference between them and level of NK&INF-γ P= 0.13, 0.46 respectively (Figure 3-3). To show the association between study variables NK &INF-γ stations of measurements (baseline, after 3 months and after 6 months) was aggregated together. As shown in (Figure 3-4). The highest concentration of NK found in the advanced stage mean was about (14.293) compared with stage-I where the mean is 8.016. There was a statistically significant (P<0.001). Meanwhile, the level of INF-γ was decreasing gradually with the stage of NSCLC. It has the lowest mean of (0.13) in stage-IV compared with stage-I where mean is about (0.229). There are statistically significant between INF-γ and staging of lung cancer(P<0.001). The study also measured the linear correlation coefficients to show how strong the relationship between two variables and staging of cancer. Also, the result that shows there is a strong positive correlation between the high level of NK cell and staging of CA there is a very strong positive relationship (r= 0.608), whereas in INF-γ there is a strong negative linear correlation that means the level decrease with a bad prognosis of NSCLC patients (r= -0.486).



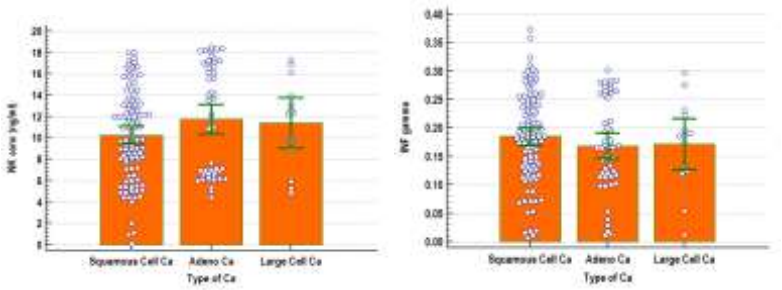
(a) NK by Gender (b) INF-γ by Gender

Figure 3-1: ADot Diagram with Error Bars Showing the Mean (with its 95% Confidence Interval) Shows the Distribution of Three Variables by Gender



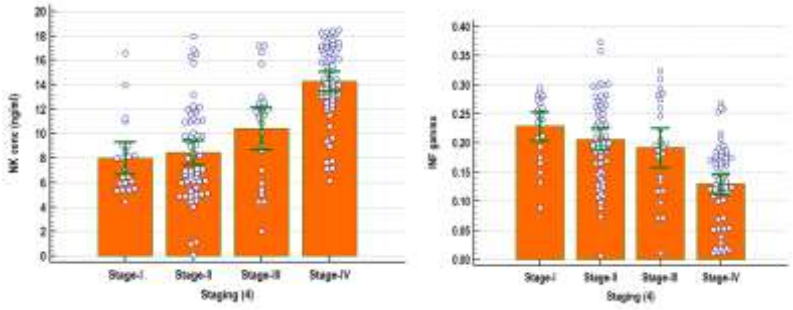
(a) NK by Age Groups (b) INF-γ by Age Groups

Figure 3-2: A Dot Diagram with Error Bars Showing the Mean (with its 95% Confidence Interval) by Age Groups



(a) NK (b) INF-γ

Figure 3-3: A Dot Diagram with Error Bars Showing the Mean (with its 95% Confidence Interval) Histopathology Phenotype of NSCLC



(a) NK (b) INF-γ

Figure 3-4: A Dot Diagram with Error Bars Showing the Mean (With Its 95% Confidence Interval) for Two Variable Levels among NSCLC Staging

The Correlation between NK and INF-γ

The measurements of NK concentration were grouped into three ordinal categories based on a quantile method. The first third of subjects with the lowest NK values constituted the first tercile (<6.6 in the current study sample), the last third of the highest measurements constituted the third or highest tercile (≥13.2), while the remaining third in the middle constituted those with average values (6.6 -13.1). As shown in (Figure 3-5), the INF-γ titer decrease with increase of NK concentration mean of INF-γ (0.1161) in the highest NK concentration compared with mean of INF-γ in the lowest NK tercile (<6.6 ng/ml) with statistically significant difference between them (NK & INF-γ conc.) and the INF-γ have a strongly negative linear correlation coefficients (r= -

0.69); that means the decrease correlated with increasing quantity of inhibitory receptor (NKG2A/CD94) NK cell.

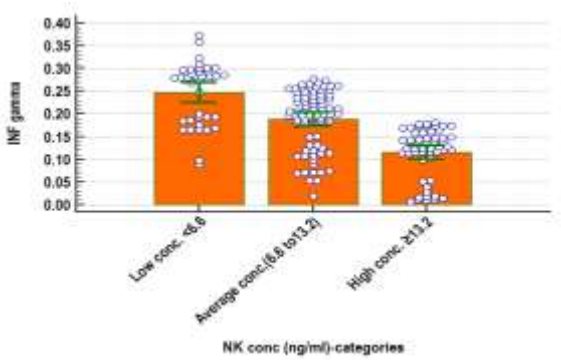


Figure 3-5: A Dot Diagram With Error Bars Showing the Mean (with its 95% Confidence Interval) INF-γ by NK Tercile Categories

Surviving Rate According to NK & INF-γ levels

The high expression of NK cell level when patients come with high concentration of NK (≥13.2) the probability of death and bad prognosis increase to (59.1%) compared with average concentration of NK (6.6 to13.2) where dying ratio about (24.1%) as appears in, (Table 3-1 and Figure 3-6), where mean of surviving time (5.2) of high concentration (≥13.2) lower than mean (5.9) of average concentration (6.6 to13.2) among six month of follow up with statistically significantly difference between them (*P*=0.004). whereas there is no surviving rate among the low concentration of NK (NKG2A/CD94). Surviving rate also measured the effects of INF-γ level on dying and surviving status in NSCLC patients, the Kaplan Meir shows that patients with high concentration (≥0.2361) of INF-γ have high chance to survive (75%) when compared with low (≤0.1780) concentration where surviving status (40%) decreased about (25%) as shown in, (Table 3-2and Figure 3-7), and according to Log-rank test the mean survival time was significantly lower (5.2 months) among cases with lowest INF-γ (≤0.1780) concentration at baseline compared to those with higher INF-γ values (mean survival increased to 6 months). Low INF-γ at baseline would predict a poor prognosis (in terms of mortality) for cases with NSCLC.

Table 3-1: Actuarial Life Table (Kaplan Mier survival) Showing the Cumulative Survival and Death Estimates (in Addition to Mean Survival Time) During a Maximum of 6 Months Follow up Period by Baseline NK -Trecile categories

NK conc (ng/ml)-First measurement-categories	Mean survival time (months)	*P* (Log rank test)
Low conc. <6.6	***	0.004
Average conc.(6.6 to13.2)	5.9	
High conc. ≥13.2	5.2	

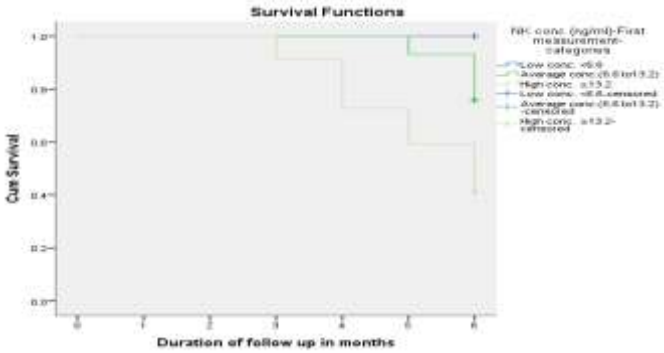


Figure 3-6: A Survival Curve Comparing the Cumulative Survival Rates during 6 Months of Follow up by NK Categories at Baseline.

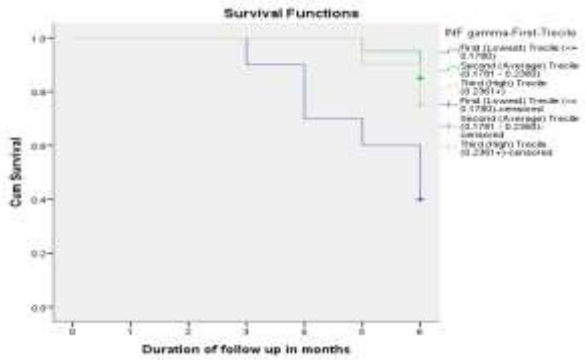


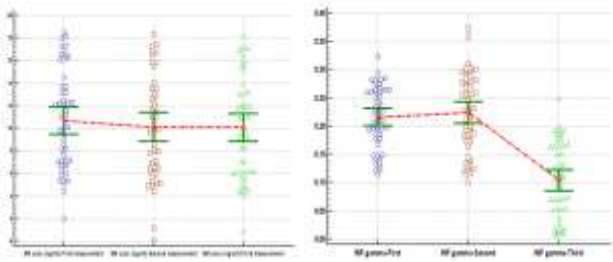
Figure 3.7: A Survival Curve Comparing the Cumulative Survival Rates during 6 Months of Follow up by INF-γ Categories at Baseline.

Table 3-2: Kaplan Mier Survival Showing the Cumulative Survival and Death Estimates (in Addition to Mean Survival Time) During a Maximum of 6 Months Follow up Period by Baseline INF- γ Trecile Categories.

INF -γ-First-Trecile	Mean survival time	P (Log-rank test)
(Lowest) Trecile ≤0.1780)	5.2	0.02
(Average) Trecile (0.1781 - 0.2360)	6.0	
(High) Trecile (≥0.2361)	5.9	

Measurement of the immunological marker after three and Six Monthof follow up post treatments

The present data show in the first time measured of NK and INF-γ three times by follow up of patients for six months and, as shown in (Figure 3-8). The concentrationof NK cell measurement as shown in (Table 3-3 and Figure 3-8), after three months of follow up, the NK concentration significantly decreased by a mean of (- 0.559) that refer to the difference between the second mean of measurements (10.852) compared with baseline level (11.411). Increasing the follow-up period to 6 months significantly decreased NK concentration by (-0.6) compared to baseline level *P*<0.001. The quantity of INF-γ changed after three month of follow up levels as appears in (Table 3-4 and Figure 3-8), increase about (0.0067) compared with baseline measurement (0.2069) with statistically significant changes *P*<0.001, then after six month of follow up the mean of INF-γ decrease (0.1118) compared with baseline (0.2161) and there are statistically significant between them *P*=0.021.



(a) NK with time (b) INF-γ with time

Figure 3-8: A Dot Diagram with Error Bars Showing the Mean (with its 95% Confidence Interval) With Time (Baseline, after three Months and after 6 Months of Follow up).

Table 3-3: Changes in NK after Three and Six Months of Follow up Compared to Baseline Measurements

	First measurement (baseline)	Second measurement (after 3 months)	Changes after 3 months	*P*	First measurement (baseline)	Second measurement (after 6 months)	Changes after 6 months	*P*
NK conc (ng/ml)			<0.001				<0.001	
Range	(2 to 18.5)	(0.13 to 18.4)	(-8.94 to 1.08)		(2 to 18.5)	(0.96 to 18.27)	(-1.95 to 1.83)	
Mean	11.411	10.852	-0.559		10.681	10.082	-0.6	
Interquartile range	(4.377 to 0.565)	(4.6 to 0.594)	(1.218 to 0.157)		(4.287 to 0.606)	(4.364 to 0.617)	(0.758 to 0.107)	
N	60	60	60		50	50	50	

Table 3-4: Changes in INF-γ After Three and Six Months of Follow up Compared to Baseline Measurements

	First measurement (baseline)	Second measurement (after 3 months)	Changes after 3 months	*P*	First measurement (baseline)	Second measurement (after 6 months)	Changes after 6 months	*P*
INFγconc. (ng/ml)			<0.001					0.021
Range	(0.114 to 0.324)	(0.1 to 0.374)	(-0.0232 to 0.138)		(0.115 to 0.324)	(0.0073 to 0.2495)	(-0.2402 to -0.0188)	
Mean	0.2069	0.2136	0.0067		0.2161	0.1042	-0.1118	
Interquartile range	(0.0547 to 0.0071)	(0.0691 to 0.0089)	(0.0218 to 0.0028)		(0.0534 to 0.0076)	(0.0645 to 0.0091)	(0.0305 to 0.0043)	
N	60	60	60		50	50	50	

DISCUSSION

Sixty patients with newly diagnosis NSCLC, thirty-seven of them with SCC and eighteen of them with the AD and only five of them with LCC included in this study. There are no statistical differences between NK, INF-γ and the histopathological types of NSCLC. Additionally, there was no significant difference between gender and levels of two variables. Meanwhile, among the age group, the present study has shown, the level of NK cell, there is a significant decrease in the level of NK $CD56^{bright}CD16^{-}$ subset with weak negative correlation coefficients (r= - 0.256 *P*<0.001); this agrees with Camous et al., (2012) who found that NK $CD56^{bright}$ $CD16^{-}$ decreases with age and low cytotoxicity. The natural killer cell is innate immune cells which have a critical role in immunosurveillance by mediating anti-cancer immune response (16). As mention previously, NK consistency is about 10-15% of peripheral blood and there are two subsets: first, $CD56^{dim}$ $CD16^{+}$ which found in 90% of peripheral blood and second $CD56^{bright}$ $CD16^{-}$constitutes about 5% of all lymphocyte in peripheral blood (17). In the present study, ELISA result show elevated NKG2A /CD94 marker in the serum of NSCLC patients and the concentration increases gradually with stages. Further, the concentration increases with a bad prognosis of patients. These results are consistent with the study ofPace *et al*., (2010) who found the lowest expression of NKG2A/CD94 in malignant pleural effusion compared with peripheral blood expression of CD94/NKG2A in cancer patients, while the study of Shen et al., (2012) has found there is no distinct difference in the level of NKG2A/CD94 between colorectal cancer patients and healthy control where mean (1.02±0.47, 1.25±0.52 *P*>0.05). The down-regulation of NKG2D and the up-regulation of NKG2A may indicate immune tolerance mechanism and facilitate metastasis in a tumor environment (8). Because CD94/NKG2A expresses in the high amount on $CD56^{bright}$ $CD16^{-}$, $CD56^{bright}$CD16- subsets in peripheral blood of NSCLC patients is elevated and these subsets are found in lymph node as predominant site and recruitment dependent on cytokines and chemokine that's secreted from cancer cell (12). So these results are supported by the study of Ali *et al*., (2014) who found a significant increase of NK $CD56^{bright}CD16^{-}$ with myeloma where mean of $CD56^{bright}CD16^{-}$ about(12.2±6.33, *P*<0.001). They are also supported by the results of alsoHoltan *et al*., (2011) who shows the high level of NK cell relatedto metastases stage compare with healthy control. While Fend *et al*., (2017) found low expression at the mRNA level of the three NK isoform activator receptors correlated with advanced NSCLC patients, their results are conflicting with observation of Klöß et al., (2015) who found that the level of NK $CD^{bright}CD16^{-}$ decrease in head and neck cancer compared with healthy control. The ability of NK cell to release cytokines is mediated by the signal generated from the interaction between NK receptors, activator or inhibitory, and the ligands expressed by infectious or transformed cells like NKG2A/CD94 which are expressed by $CD56^{bright}CD16^{-}$ and act as a regulator for NK activity through binds with inhibitory ligand (24). The heterodimer form of CD94/NKG2 is a C-type lectin receptor, consisting of the covalent bond linked with CD94, and NKG2 protein consisting ofintra, short and non-signaling protein. There are several types of NKG2A, B, C or E. Inside NK cell and there are molecules named immune-receptor tyrosine-based inhibition motifs (ITIMs), link with surface KNG2A/CD94 receptors. Hence when binding occurs between NKG2A and their receptors, the inhibitory signal formed through this motif help to regulate the activation of NK cells. After binding between CD94/NKG2A and non-classical HLA-E, molecules lead to activate the inhibition of NK cell (25,26). The elevated NK cell in peripheral blood is inefficient in a form its level increases with advanced stage because of mechanisms used by the tumor to escape from the NK cell response. One of these mechanisms is done when the cancer cells can increase the expression of MHC class I type E molecules to inhibit NK cell cytotoxic functions (27,28). Another mechanism that it down-regulate activator receptor ligand like NKG2D ligands to escape NK recognition. Also, tumor secretes inhibitory cytokines that help in an increase the activity of T- reg cells or myeloid-derived suppressor cells, which makes the tumor environment highly suppressive and limits the efficacy of NK anti-tumor functions (29). High quantity of NK cell$CD56^{bright}CD16^{-}$ in peripheral blood in an advanced stage with an expression of NKG2A/CD94 reflecting low cytotoxicity of NK cell (25). INF-γ shows an important role in protecting against transformed cell and tumor growth (30). Level of INF-γ was decreasing gradually with the stage of NSCLC. It has the lowest mean (0.13) in stage-IV compared with stage-I where mean about (0.229). There is a statistically significant difference between INF-γ and staging of lung cancer (*P*<0.001). Such results are consistent with Lee et al., (2013) who found that low serum of IFN-γ level was significantly associated with tumor stage in liver cancer patient, and the study of Lee, Park, *et al.*, (2017) who explored level of IFN-γ in gastric cancer patients with distant metastasis and found significantly lower in IFN-γ levels than patients with stage-II or III (*P* = 0.001). Interestingly, IFN-γ levels in gastric cancer patients with stage-I were also significantly lower than those of healthy donors (*P* <0.001) andConti-Freitas et al., (2012) show that IFN-γ levels produced by the preoperative period and the late postoperative period cultures were lower than the levels produced by the control group cultures (*P* = 0.001). The study also measured the linear correlation coefficients to show how strong the relationship between INF-γ and staging there is a strong negative linear correlation. Results are consistent with (31) who found that the level of INF-γ correlated with more advanced tumor stage. In early-stage INF-γ plays a role in anticancer through the activation of apoptosis pathways; after binding with receptors its act to regulate apoptosis-related genes and also increase cell sensitivity to apoptosis through activating transformed cell to express MHC-I (34). Moreover, it can activate immune cells like T-helper cell responses and macrophages leading to increasing the production of pro-inflammatory cytokines (33,35). In the advanced stage the immune system response weak as a result of an increase in inhibitory mediators and tumor

growth factors that leads to decrease in INF-γ levels (36). The study also investigates the relationship between the level of NKG2A/CD94 and secretion of INF-γ, and the level of INF-γ reflecting the cytotoxicity of NK-cell, NKG2A/CD94 receptors when expressed in a high level on $CD56^{bright}$ $CD16^{-}$, it can prevent activation and secretion of INF-γ from NK-cell subset (37). The INF-γ titer decreases with the increase of NK concentration means of INF-γ (0.1161) in the highest NK concentration compared with a mean of INF-γ in lowest NK (<6.6 ng/ml) with the statistically significant difference between them (NK & INF-γ conc.). The INF-γ have strongly negative linear correlation coefficients (r = - 0.69); that means the decrease is correlated with the increasing quantity of inhibitory receptor (NKG2A/ CD94) NK cell. Kaplan-Meier estimates that the NK cell level when patients come with high concentration, the probability of death and bad prognosis increases compared with low concentration. This result consist with other datathat show that the high expression of NKG2A is associated with poor prognosis of patients with hepatocellular cancer (*P*=0.0015) (37) and, with study that found the survival time of lung cancer patient was positively related to NK cell infiltration degree in lung cancer. Thus, the down-regulation of NKG2D and the up-regulation of NKG2A may indicate immune tolerance mechanism and facilitate metastasis in the tumor environment (38). Meanwhile, the other result shows, that the overall survival and disease free survival were not significantly different in NSCLC patients having high and low NK cell infiltrations (39). These findings are in accordance with the fact that intratumoral NK cells exhibit a strong down-regulation of activating receptors that are important for tumor cell recognition and killing and display impaired capacities to stimulate degranulation. Indeed, the clinical outcome of patients would be more dependent on NK cell phenotype and functionality rather than on NK cell density. In INF-γ level when compared with the surviving rate. The lowest INF-γ concentration at baseline compared to those with higher INF-γ values (mean survival increased to 6 months). Low INF-γ at baseline would predict a poor prognosis (in terms of mortality) for cases with NSCLC. Such results agree with the other study of that shows that the survival differences between the carriers of the different genotypes were statistically significant with log-rank *P* values of 0.04, 0.03 and 0.04, respectively in patients withcolorectal cancer (40). Meanwhile, other researchers found that death ratio increases with rectal cancer patients having genetic variation lead to defects in INF-γ secretion and progression free survival and response to treatment was significantly longer in NSCLC patients with high versus low IFN-γ expression (41). Moreover, some data show also progression-free survival was significantly longer in melanoma patients with high versus low IFN-γ expression and the progression free survival increased when used treatment induced INF-γ secretion with advanced NSCLC and overall survival (42). The data show for the first time that variables (NK, and INF-γ) were measured three times during follow up for six months. In the NK cell measurement, after three months of follow up, the NK concentration significantly decreased by a mean of (0.559) compared to the baseline level. Increasing the follow-up period to 6 months significantly decreased NK concentration by (0.6) compared to baseline level *P*=0.001. In pre-treatment, the levels of NK cells elevated, and decline in levels when checked again after three months of treatments. The changes may be as a result of a response to treatments. While the quantity of INF-γ changed after three months of follow up levels increased about (0.0067) compared with baseline measurement (0.2069) with statistically significant changes *P*<0.001. Then, after six months of follow up, the mean of INF-γ decrease (0.1118) when contrast with first measured (0.2161) and with a significant difference between them *P*=0.021 (31).

CONCLUSION

High expression of NKG2A/CD94 of $CD56^{bright}$ $CD16^{-}$ in peripheral blood of NSCLC patients play an important role correlated with bad prognosis which may be used as the prognostic marker. High level of INF-γ refers to the good prognosis of NSCLC patients and vice versa.

AUTHOR CONTRIBUTIONS

All authors contributed to this manuscript. Nasser Ghaly Yousif conducted the study design, Samar Muayad Mohammed, Kareem Ghaly Mohammed, Fadhil G. Alamran and Najah Rayish Hadi conducted the interpreted the data. Song Zheng, Maitham Ghaly Yousif, Jillen Lee and Jonthan Adrienne wrote the original manuscript and reviewed and edited the manuscript. All authors read and approved the final manuscript.

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