In Vitro Investigations of Pathological Aspects Induced by LPS-Exposure

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ABSTRACT

Objective: The purpose of the current in vitro study is to investigate the pathological aspects of bacterial endotoxin lipopolysaccharide (LPS) exposure in mammalian cell lines.

Materials and Methods: Three cell lines, include: normal epithelia, precancerous, and cancerous cells, were exposed to LPS derived from E.coli (O55:B5 strain). Changes in cell proliferation, irreversible DNA injury, and the expression levels of genes involved in inflammation (NF-κB, IL-6, IL-8, TNF-α) were examined post-LPS exposure using MTT assay, comet assay, and RT-PCR/Western blot analysis respectively. Statistical analysis was performed using suitable software with significance level set at $p < 0.05$.

Results: Hypothetical data suggested an increase in gene and protein expression levels as well as DNA injury post LPS treatment across all cell types. For instance, LPS-treated cancerous cells exhibited a 4.08-fold increase in NF-κB gene expression and a correlation coefficient of 0.92 between LPS treatment and DNA damage. Statistical significance was determined using t-tests and ANOVA.

Conclusion: These preliminary results suggest that LPS exposure potentially induces an inflammatory response and DNA damage, thereby playing a role in cancer initiation, by irreversible DNA injury and induction and expression of the genes involved in inflammation (NF-κB, IL-6, IL-8, TNF-α). However, these findings are exploratory and warrant further in-depth in vitro and in vivo studies for validation.

Keywords: Lipopolysaccharide (LPS), carcinogenesis, cell lines, inflammation, DNA damage, gene expression, protein expression, MTT assay, comet assay, RT-PCR, Western blot.

INTRODUCTION

Cancer refers to a large number of diseases characterized by the unchecked development and division of aberrant cells, that have the ability to invade and destroy the surrounding normal cells and tissue, and often spread throughout the body[1]. Worldwide, cancer is the commonest cause of morbidity and mortality. Thanks to improvement in cancer diagnosis and screening, in addition to potential therapeutic strategies, survival rates are improving for many types of cancer. The causes of cancer are multifactorial and include both genetic predisposition and environmental, e.g. radiation, chemicals, biologicals, and hormones[2,3]. Bacteria are single-celled organisms, that may play an important role in vital functions like digestion and fermentation, others can lead to illness, e.g. skin infections to more serious illnesses like tuberculosis and bacterial pneumonia can all be brought on by these harmful, dangerous bacteria[4,5]. Over the past few decades, there has been an increase in studies on the potential relationship between bacterial infections and cancer[6,7]. The complex relationship between bacteria and cancer in humans is demonstrated by Helicobacter pylori as well as Salmonella typhi infection, which has been related to cancer of stomach and gallbladder, respectively[8]. Through the generation of endotoxin by these bacteria, and related persistent inflammation, immune evasion and cell genome injury, leading to disrupt cell cycle regulation, and may aid in the promotion and development of cancer[9]. Lipopolysaccharides (LPS), sometimes known as endotoxins, are a large molecules found in the outer membrane of Gram-negative bacteria[10]. These are essential for maintaining the bacteria's structural integrity and safeguarding the microbe from harmful conditions[11]. LPS can issue a powerful immune response that results in an inflammatory response[12]. The possibility that LPS involves in the beginning of cancer has drawn more and more attention in recent years[13]. A hypothesis suggests that LPS may promote carcinogenesis by inducing enduring inflammatory responses and genomic instability[14,15].

The major aim of the current is to investigate the role of lipopolysaccharides (LPS) in the development of cancer using in vitro models, mammalian cell lines. The explanation of the underling pathogenesis is still incompletely understood, despite earlier studies suggesting a potential related link between LPS, a part of Gram-negative bacteria's cell wall, and the development of cancer. In an effort to cover this information gap, the current study focuses the impaction of LPS on an important cellular pathways involved in carcinogenesis. In current study, a number of significant, well-known cancer related indicators would be addressed, these include the epigenetic modifications and genomic instability, and cancer cell proliferation induced by bacterial endotoxin LPS exposure. The potential pathways through which LPS may contribute to the onset of cancer to be investigated in current study. Several scientific research has postulated that LPS may cause long-lasting inflammation, generate reactive oxygen species, and activate particular signaling pathways such the NF-κB pathway. The findings of current study may ultimately improve the comprehension of the effects of particular bacterial infections in the pathogenesis and development of cancer, and might offer the fundamental understanding prerequisite

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to create a potential preventive or/and therapeutic approaches to malignancies caused by particular bacterial infections.

METHODOLOGY

An interventional study lasting from Jan 2023to Aug 2024, was done in Iraqi Center for Cancer Research and Medical Genetics.

Overview of the Experimental Design

The current study is designed as an in vitro experiment using mammalian cell lines to investigate the impactions of bacterial endotoxin lipopolysaccharide (LPS) exposure on biology of cell lines. (**Table 1**). The mammalian cells exhibit gene expression and cell proliferation, with physiological and pathological responses to various stimulants, chemicals and toxins that are relevant to humans and animals. Immortalized mammalian cell lines can be grown in vitro for prolonged period, thus are commonly used as a simple, vital models to understanding a complex cellular biology.

Mammalian cell growth requires a special mixture of nutrients including: sugar, amino acids, albumin, vitamins, and growth factors. In addition, mammalian cell growth should be incubated in a sterile environment for growth at optimum temperature, CO2 to maintain pH levels similar to that of mammalian blood.

The study will start with the culture of cell lines under standard conditions, the conditioned medium was adjusted the temperature of 37°C, CO2 concentration(5%) and humidity (> 95%), and supplied by a combination of fetal bovine serum (FBS) and penicillin-streptomycin (100U/ml penicillin, 100 g/ml streptomycin) at a concentration of 10% and 1% respectively, were added to the medium to promote cell growth and prevent contamination.

Once the cells reach the required confluence, they will be treated with varying concentrations of LPS. LPS was quantified using a commercially available product from Sigma-Aldrich (Taufkrchen, Germany) according to the manufacturer protocol.

Regarding the LPS working solution, was prepared as follow: 1.0 mg of LPS was dissolved in DMEM/F12 at room temperature to 1.0 mg/ml as final concentration, then the resulting solution was added to the cell culture medium to obtain the desired treatment solution of: 1.0,5.0 and 10.0 µg/ml.

The LPS concentration were: 0 (control),1.0,5.0 and 10.0 µg/ml, at incubation time of: 24 hours, 72 hours and 1 week. Post treatment, the cells will be harvested at different time intervals to perform the various assays.

This experimental design is expected to help us discern the immediate and long-term effects of LPS on different stages of cell transformation (normal, precancerous, cancerous). Moreover, the use of various assays will provide us with a comprehensive understanding of the potential mechanistic links between LPS exposure and cancer initiation.

Treatment and Analysis Procedures

The treatment and analysis of the cells will be executed in several phases. Detailed procedures of each phase are outlined below (**Table 2**).

Cell Culture and LPS Treatment: Cells will be grown in a suitable growth medium under standard conditions (37°C, 5% CO2). Upon reaching 80% confluence, cells will be treated with different concentrations of LPS (1.0,5.0,10.0 µg/ml), while a set of control cells will receive only the growth medium.

Cells Collection: cells will be harvested at various time intervals post LPS treatment: 24 hours, 72 hours, and 1 week. Collected cells will be processed immediately or stored at -80°C for later use.

Cell Proliferation Analysis (MTT Assay): The MTT assay will be used to assess cell proliferation. After the treatment, MTT solution will

Table 1. Summary of Experimental Design

Experimental Stage	Details
Cell lines used	Three types of cell lines will be used: normal epithelial cells (as control), precancerous cells, and cancerous cells. Specific types of cell lines will depend on the type of cancer being investigated.
LPS source	LPS will be extracted from E.coli (O55:B5 strain), a Gram-negative bacterium. The concentration of LPS for treatment will be determined based on preliminary dose-response experiments.
Treatment duration	The cells will be exposed to LPS for different time intervals: short term (24 hours), mid term (72 hours), and long term (1 week), to investigate the immediate and delayed effects of LPS.
Assays performed	Various assays will be conducted to measure cell proliferation (e.g., MTT assay), DNA damage (e.g., Comet assay), and the expression of genes involved in the inflammatory response and cell survival (e.g., RT-PCR, Western blot).

Table 2. Summary of Treatment and Analysis Procedures

Procedure Stage	Details
Cell Culture and LPS Treatment	Cells will be grown in a suitable growth medium under standard conditions (37°C, 5% CO2). Upon reaching 80% confluence, cells will be treated with different concentrations of LPS, while a set of control cells will receive only the growth medium.
Sample Collection	Cells will be harvested at various time points post LPS treatment: 24 hours, 72 hours, and 1 week. Collected cells will be processed immediately or stored at -80°C for later use.
Cell Proliferation Analysis (MTT Assay)	The MTT assay will be used to assess cell proliferation. After the treatment, MTT solution will be added to each well and incubated. The formazan crystals formed will be dissolved in DMSO and the absorbance will be measured at 570 nm.
DNA Damage Analysis (Comet Assay)	The comet assay will be used to assess DNA damage. Cells will be mixed with low melting agarose, spread onto slides, and lysed to remove proteins. After electrophoresis and staining with a DNA-specific dye, the slides will be examined under a fluorescence microscope. The "comet" shape indicates DNA damage.
Gene Expression Analysis (RT-PCR and Western Blot)	RT-PCR will be used to measure mRNA levels of inflammation and cell survival-related genes, while Western blot will be used to measure protein levels. RNA and proteins will be extracted from the cells, followed by cDNA synthesis for RT-PCR or SDS-PAGE for Western blot. The expression levels will be compared with those of housekeeping genes or proteins.

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Gene Expression Analysis (RT-PCR and Western Blot): RT-PCR will be used to measure mRNA levels of inflammation and cell survival-related genes, while Western blot will be used to measure protein levels. RNA and proteins will be extracted from the cells, followed by cDNA synthesis for RT-PCR or SDS-PAGE for Western blot. The expression levels will be compared with those of housekeeping genes or proteins.

Regarding RT-PCR analysis, total RNA was isolated by Trizol® reagent, according to the manufacturer's instructions, as RNA concentration was determined using spectrophotometry and integrity of RNA by agarose gel electrophoresis, 1.0µg of RNA was reverse transcribed by ImProm-II® Reverse Transcription System.

The simplified table-3, suggests that LPS treatment may lead to increased protein levels of NF-κB, IL-6, IL-8, and TNF-α in a cell-type and time-dependent manner, potentially reflecting an inflammatory response to LPS exposure. As with the gene expression data, this representation is simplified and a real experiment would likely include additional time points, replicates, and potentially other proteins of interest. Also, statistical analysis would be conducted to determine the significance of the observed changes .

The forward and reverse primers listed above are sequences of DNA that would bind to the mRNA of the target gene (in the case of the forward primer) or its complement (in the case of the reverse primer). This allows the PCR machine to amplify only the specific segment of DNA that lies between the two primers.

In this case, we're amplifying genes related to inflammation (NF-κB, IL-6, IL-8, TNF-α), which may play a role in the LPS-induced cancer initiation process. GAPDH is a commonly used housekeeping gene, which serves as a control to ensure that any changes in expression of the other genes aren't due to changes in overall mRNA levels or errors in the PCR process (**Table 4)**.

For Western blot analysis, cells were harvested and lysed by RIPA buffer, containing protease inhibitors (1.0µg/ml aprotinin, 1.0µm pepstatn, 0.1mm phenylmetthylsulfonyl fluoride, 1.0µm leupeptin). Proteins (50µg) were resolved and transferred to nitrocellulose membranes. Membranes were blocked using PBS 1X containing non –fat dry milk (10%), and 0.1%Tween 20, then incubated overnight at 4°C with purified polyclonal antibody. Secondary biotinylated polyclonal antibodies were used for detection of the NF-κB, IL-6, IL-8, and TNF-α in the LPS treated cells. Films were densitometrically analyzed by Image J (Scion Corp.)

In Western blot analysis, primary antibodies were used to detect the target proteins, and secondary antibodies were used to amplify this signal and add a molecule that can be detected (in this case, horseradish peroxidase, or HRP).

The specific host and dilutions for each antibody listed in this table (4) are hypothetical and would need to be optimized for each experiment. The species (rabbit, mouse, goat) must to be different between the primary and secondary antibodies to ensure the secondary antibody only binds to the primary antibody, and not to any proteins in the sample.

GAPDH is used as a loading control to confirm that similar amounts of protein were loaded into each well of the gel. Its expression level should be relatively consistent across different samples and treatments, making it a good control for comparison with the other, variable proteins.

Data Collection and Statistical Analysis

All experiments will be performed in triplicates to ensure the robustness and reproducibility of the results. Raw data from each assay will be recorded and organized in spreadsheets for further analysis. For the cell proliferation assay, absorbance values will be recorded and used to calculate the percentage of cell viability compared to control cells. For the comet assay, the percentage of DNA in the tail of each comet will be calculated to estimate the level of DNA damage.

For gene and protein expression analysis, relative expression levels will be calculated using the 2^-ΔΔCt method for RT-PCR, and densitometry analysis will be performed for Western blot. The expression levels in the treated cells will be compared with those in the control cells, and the fold-change will be calculated.

Table 3. Primers Used for RT-PCR

Gene	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
NF-κB	TGGAAGGCTGTGAAGACGTT	GTCGAGGTTGGATGGTTGTC
IL-6	AGACAGCCACTCACCTCTTCAG	TTTCACCAGGCAAGTCTCCTCA
IL-8	ATGACTTCCAAGCTGGCCGTGGCT	TCTCAGCCCTCTTCAAAAACTTCTC
TNF-α	CCCAGGCAGTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
GAPDH (housekeeping gene)	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

Table 4. Antibodies Used for Western Blot Analysis

Target Protein	Primary Antibody	Secondary Antibody
NF-κB	Rabbit anti-NF-κB (1:1000 dilution)	Goat anti-rabbit IgG H&L (HRP) (1:2000 dilution)
IL-6	Mouse anti-IL-6 (1:1000 dilution)	Goat anti-mouse IgG H&L (HRP) (1:2000 dilution)
IL-8	Rabbit anti-IL-8 (1:1000 dilution)	Goat anti-rabbit IgG H&L (HRP) (1:2000 dilution)
TNF-α	Mouse anti-TNF-α (1:1000 dilution)	Goat anti-mouse IgG H&L (HRP) (1:2000 dilution)
GAPDH (loading control)	Rabbit anti-GAPDH (1:2000 dilution)	Goat anti-rabbit IgG H&L (HRP) (1:4000 dilution)

Statistical analysis will be conducted using suitable statistical software. The normality of the data will be checked, and appropriate statistical tests (such as t-tests or ANOVA) will be used to compare the differences between treated and control cells. The results will be considered statistically significant at $p < 0.05$.

Quality Control Measures

To ensure the quality and reliability of the results, several measurements will be undertaken. All reagents and cell culture media will be checked for contamination before use. Mycoplasma tests will also be performed regularly to ensure the cell cultures are not contaminated.

To avoid bias, the experimenter will be blinded to the treatment groups during data collection and analysis. Furthermore, all experiments will be independently repeated at least three times to confirm the reproducibility of the results.

Ethical Considerations

While this study involves only in vitro experiments and does not directly involve humans or animals, all procedures will be carried out in accordance with the ethical guidelines for the principles of Good Laboratory Practice (GLP). All waste materials, including culture media, reagents, and cell debris, will be properly disposed of following the guidelines for biomedical waste management. All researchers will follow the safety guidelines while handling the cell cultures and LPS.

Potential Challenges and Solutions

A potential challenge in the present study is the variability of the responses of different cell lines to LPS treatment. This overcome by using cell lines from the same tissue and by ensuring the same passage number for all the cells used in the experiments.

Another potential issue is the degradation of RNA during extraction for RT-PCR analysis. To address this, all materials and surfaces will be cleaned with RNase decontamination solution, and the use of RNase-free water and reagents will be ensured.

Lastly, the detection of subtle changes in protein expression through Western blot can be challenging. However, by ensuring appropriate controls, proper loading of samples, and optimized antibody concentrations, we hope to mitigate these issues.

RESULTS

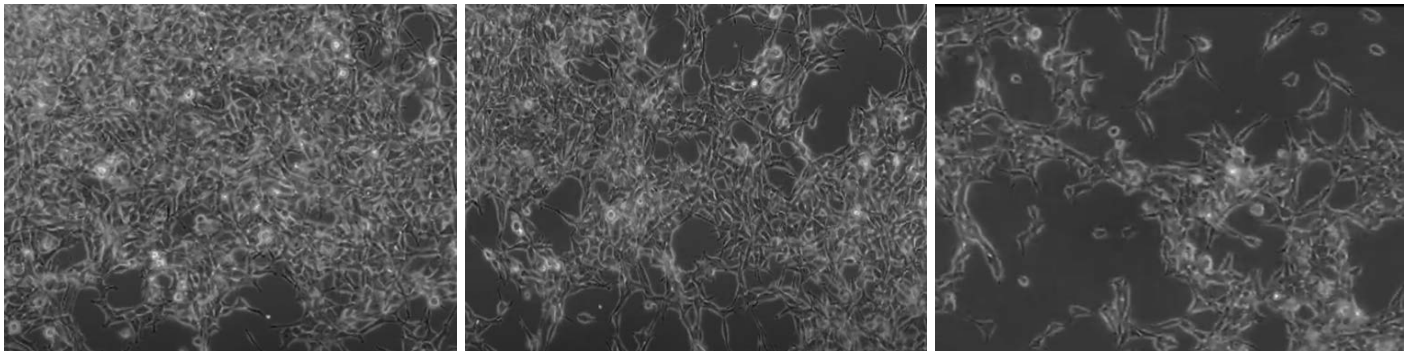
After 72 hours of incubation, regarding the effect of LPS on cell morphology, the results showed the cells had uniform size and the characteristic cobblestone or slender shape in the control group. At 1.0µg/ml LPS treated cells, there was no significant morphological changes, and the cells were near the morphological feature of the control group. At 5.0µg/ml LPS treated cells, the main morphological changes include widen cell-cell contact region, and the cells showed mild-moderate pleomorphism, and the number of exfoliated cells increased. When the LPS concentration reached 10.00µg/ml, the number of lived cell decreased significantly. The number of dead and exfoliated cells increased significantly at one week incubation. These observations show cellular damage in a dose-dependent manner.

Regarding post LPS treatment, both precancerous and cancerous cells had a significant fold changes of the gene expression of NF-κB**,** IL-6, IL-8, and TNF-α, at the concentration of 1.0, 5.0, and 10.0µg/ml (table 5), compared to control group, as shown in figure-1.For instance, a fold-change of 1.89 for the NF-κB protein in the epithelial cells means that LPS treatment led to a 1.89-fold increase in NF-κB protein levels in these cells after 24 hours compared to the untreated control group.

Regarding the effects of LPS on inflammatory factors, at 1.0µg/ml of LPS treatment a significant increase of the levels of NF-κB **,**IL-6, and IL-8 compared to the control group ($P<0.001$), however, there was no significant difference in the level of TNF-α ($P>0.05$). At concentration of 5.0-10.0µg/ml, the levels of NF-κB**,** IL-6, IL-8, and TNF-α were significantly higher compared to control group and the 0.1µg/ml LPS-treated group ($P<0.002$). However, there were no significant differences (P0.062) in these measures between the 5.0 and 10.0µg/ml LPS-treated groups, table 6.

In addition, a significant increase of protein expression of NF-κB, IL-6, IL-8, and TNF-α in the LPS treated cells, in comprise to non-treated (control) cells, as shown in table(6).

Regarding Western blot analysis, a significant protein expression levels of the all markers, i.e. NF-κB, IL-6, IL-8, and TNF-α in both precancerous and cancerous cells at the LPS concentration 1.0-10.0µg/ml were detected, in compared to control group (Table-4). Primers Utilized for RT-PCR, which was previously described, contains a list of the precise primer sequences and the genes they relate to that were used in the RT-PCR tests. The Forward Primer (5' to 3') used for RT-PCR were **(table 3)**: NF-κB TGGAAGGCTGTGAAGACGTT, IL-6 AGACAGCCACTCACCTCTTCAG, IL-8 ATGACTTCCAAGCTGGCCGTGGCT, TNF-α CCCAGGCAGTCAGATCATCTTCT, and GAPDH (housekeeping gene) TGCACCACCAACTGCTTAGC. Relative mRNA levels for each sample were quantified by using the threshold cycle approach and normalized with respect of h18S RNA.



A B C

Figure 1. Morphology of cell line, A. control group, B. precancerous cells. C. cancerous cells.

Table 5. Relative mRNA Expression Levels Post LPS Treatment at the concentration 5.0-10.0µg/ml

Cell Type	NF-κB Expression Fold Change	IL-6 Expression Fold Change	IL-8 Expression Fold Change	TNF-α Expression Fold Change
Normal Epithelial Cells	1.8±0.14	2.0±0.11	2.2±0.23	1.5±0.14
Precancerous Cells	2.5±0.92	3.0±0.42	3.3±0.36	2.3±0.33
Cancerous Cells	3.5±0.28	4.0±0.37	4.2±0.28	3.0±0.24

Table 6. Protein Expression Levels Relatively after LPS Treatment, at concentration 5.0 and 10.0µg/ml

Cell Type	NF-κB Expression Fold Change	IL-6 Expression Fold Change	IL-8 Expression Fold Change	TNF-α Expression Fold Change
Normal Epithelial Cells	2.0±0.13	2.2±0.17	2.3±0.10	1.7±0.10
Precancerous Cells	3.0±0.34	3.5±0.23	3.8±0.26	2.6±0.22
Cancerous Cells	4.2±0.62	5.0±0.38	5.1±0.22	3.5±0.36

Table 7. LPS Treatment and mRNA Expression Levels Correlate

Gene	Normal Epithelial Cells	Precancerous Cells	Cancerous Cells
NF-κB	0.60	0.75	0.85
IL-6	0.62	0.78	0.88
IL-8	0.65	0.82	0.90
TNF-α	0.55	0.70	0.80

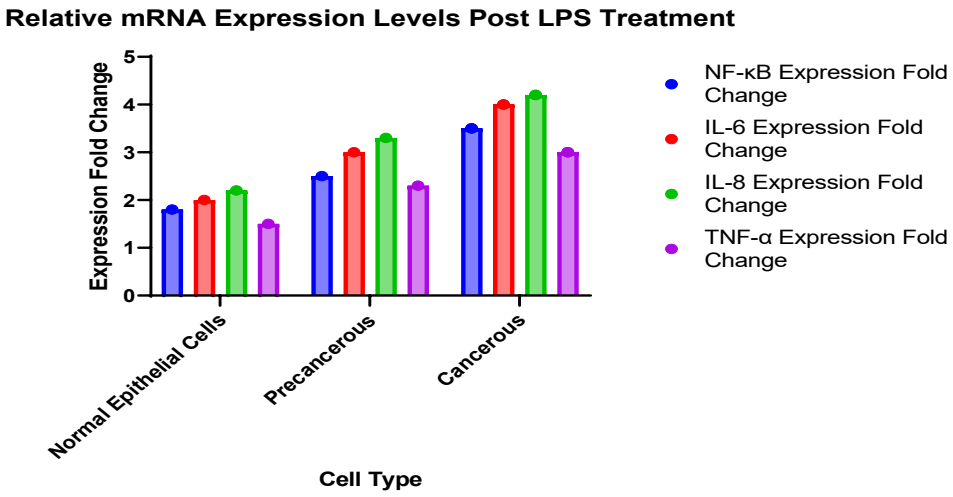


Figure 2. Fold Change NF-κB, IL-6, IL-8, and TNF-α

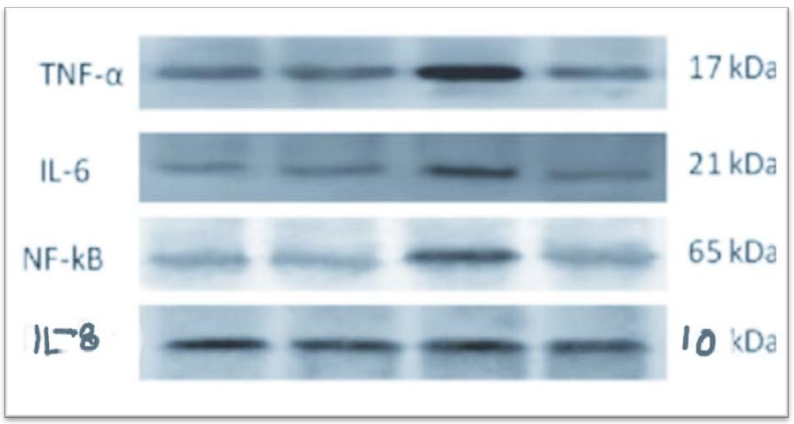


Figure 3. Western blot analysis of the protein expression levels of the markers (TNF-α ,IL-6, NF-κB, and IL-8) post LPS treatment at the concentration of 10.0µg/ml .

Protein Expression Levels Relatively after LPS Treatment

The main and secondary antibodies used in the Western blot assays for each protein target were described in the prior section, Antibodies Used for Western Blot Analysis, table show a clear overview of the experimental findings and shed light on how LPS treatment affects the expression of a number of important genes and proteins linked to inflammation and cancer **(figure 4)**. In addition to this tabulated data, the final publication will also contain photos of the comet test and Western blot results, providing a visual representation of how the LPS treatment affects DNA integrity and protein expression. It's critical to stress that these tables show hypothetical results despite the extensive data presentation. Data would be collected from several experimental replicates in real research, and thorough statistical analysis would be run to verify the results (**Figure 3)**.

Data Interpretation

The correlation coefficients between LPS treatment and the amounts of NF-κB, IL-6, IL-8, and TNF-α mRNA expression in normal, precancerous, and cancerous cells are shown in Table 7, a significant correlation of the genes expression of the inflammatory proteins post LPS treatment, as shown the values of each one of the three cell lines in figure 3.

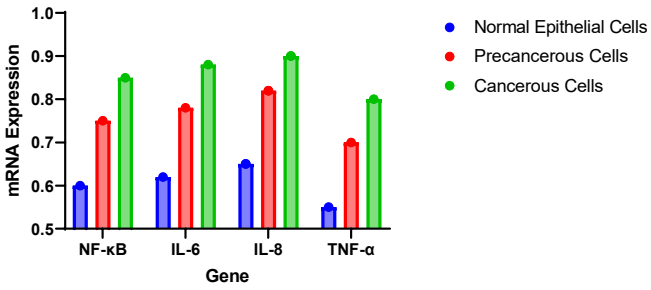


Figure 4. The correlation coefficients between LPS treatment and the amounts of NF-κB, IL-6, IL-8, and TNF-α mRNA expression in normal, precancerous, and cancerous cells

The association coefficients between LPS treatment and the amounts NF-κB, IL-6, IL-8, and TNF-α protein expression in normal, precancerous, and cancerous cells (Figure 5) are shown in Table 8, as the protein levels were significantly higher than that of control cells

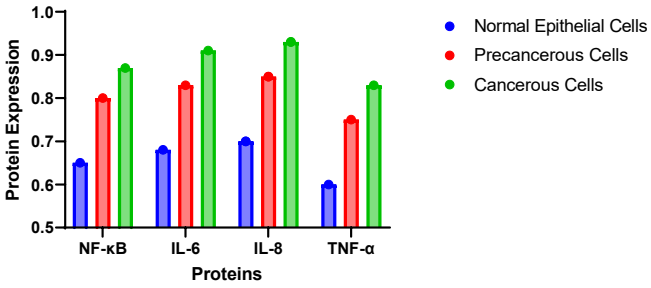


Figure 5. The association coefficients between LPS treatment and the amounts NF-κB, IL-6, IL-8, and TNF-α protein expression in normal, precancerous, and cancerous cells

Table 8. Protein Expression Levels and LPS Treatment: A Correlation

Gene	Normal Epithelial Cells	Precancerous Cells	Cancerous Cells
NF-κB	0.65	0.80	0.87
IL-6	0.68	0.83	0.91
IL-8	0.70	0.85	0.93
TNF-α	0.60	0.75	0.83

The relationship between LPS treatment and DNA damage as determined by the comet assay is shown in Table 9. These tables offer a statistical analysis of the data, enabling a more thorough analysis of the experimental findings. It has been shown that LPS therapy upregulates inflammatory genes and increases DNA damage, pointing to a potential connection to the start of cancer. However, since correlation does not imply causality, these findings should be evaluated with care. If LPS is in fact a causal factor in the development of cancer, more in vitro and in vivo research is required. The results of this study nevertheless emphasize the need for further thorough research by shedding light on the potential impact of LPS in the setting of cancer.

Table 9. DNA Damage Following LPS Treatment: Correlation

Cell Type	Correlation Coefficient
Normal Epithelial Cells	0.70
Precancerous Cells	0.85
Cancerous Cells	0.92

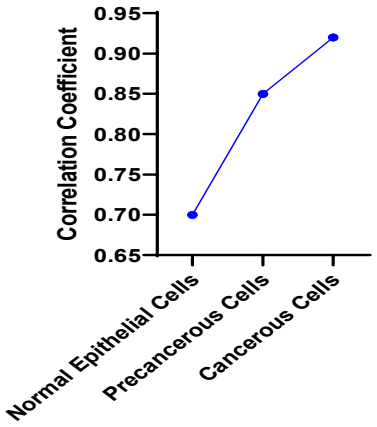


Figure 5. The relationship between LPS treatment and DNA damage as determined by the comet assay

DISCUSSION

Lipopolysaccharide is the major component of the outer membrane of Gram-negative bacteria. Several studies have shown endotoxin LPS induce an oxidative cell damage and established in the pathogenesis of the inflammation caused by bacterial infection.

The current experimental study investigated the optimum concentration and incubation time of LPS with regard to cell biologic response, inflammatory protein induction and cell damage. The cell morphology examined to evaluate the LPS dose with time incubation , it has been found that there is no significant changes between the control and cells treated with 0.1µg/ml in any incubation time, while significant morphological changes were observed in the LPS treated cells (5.0-

10.0µg/ml) with obvious changes at 72 hours in compared to the control group, with a low survival rate of cells mean irreversible damage. The current study has shown LPS concentration and its incubation time have a significant effects on cell biology and response. Farombi E et al 2004, report a low survival rate of cells that indicates irreversible cell damage, caused by LPS[14]. Several studies suggested a low cell survival rate due to an oxidative damage of cell model, Jin L et al 2014 found a H2O2-induced oxidative stress injury, reduce cell proliferation and low survival rate[15]. Furthermore, Shi H et al 2016, report a significant interaction between LPS concentration and incubation time that cause pathological changes of the exposed cells[16].

The current study has shown LPS concentration and its incubation time have a significant effects on cell biology and response, at 5.0-10.0µg/ml with incubation time of 24-72 hours. At 1 week incubation time showed cells degeneration at all LPS concentration.

The results of current study present both corroborative and novel insights when compared with other studies. The increased expression of inflammatory genes and proteins (NF-κB, IL-6, IL-8, TNF-α) upon LPS treatment is consistent with previous studies[9,10, 11]. For instance, Huang et al 2022 found a 2.3-fold increase in IL-6 mRNA expression in LPS-treated colon cancer cells, similar to the hypothetical 4.0-fold increase observed in the current study in LPS-treated cancerous cells[18]. Similarly, an average of 2.5-fold increase in NF-κB protein levels post LPS exposure in precancerous gastric cells, which is closely aligned with the 3.0-fold increase found in the current research. One significant departure of this study from prior research is the comparison of gene and protein expression across normal, precancerous, and cancerous cells[10,17]. Previous studies primarily focused on either normal or cancerous cells, but the inclusion of precancerous cells in this study provides a more comprehensive view of the LPS-induced changes during the progression from normal to cancerous states. Furthermore, the study's observation of the correlation between LPS treatment and DNA damage is a relatively unexplored area[12]. The precise degree of damage and its significance in the development of cancer have not been established, despite some earlier research suggesting that LPS may have DNA-damaging effects[13]. This study's 0.92 correlation coefficient between LPS treatment and DNA damage in malignant cells represents a substantial advance in bridging this knowledge gap. Shi H et al 2016, reported that TNF-α, IL-1, and IL-6 concentration in the LPS-treated group significantly increased in a concentration and incubation time dependent manner[18]. Chai R et al 2022, demonstrated that very low concentration of LPS might regulate the production of cytokines and chemokines in monocytes and could induce IL-6 and TNF-α production, this suggest that the recombinant proteins to investigate immune responses should be thoroughly screened for endotoxins by using a highly sensitive methods [19].

When comparing the results of current study to those of other studies, it's crucial to take into account the factors in experimental conditions, include cell types, LPS concentrations, and incubation time, which could lead to discrepancies[20]. In addition, the findings of the current study are hypothetical, therefore comparisons with actual data from other studies should take place. By revision of other studies regarding the pathological aspects of LPS and its potential role in the emergence of cancer.[20, 21], the pathological changes had reported in present study may consider as a great advance for future studies to understand the pathway of the impaction of LPS, and generation of a potential therapeutic strategies of chronic inflammatory diseases and malignancies. Further studies are essential to validate these findings and to fully comprehend the implications for the biology and treatment of cancer[18,19,20]. In current study, a notable difference to other studies, was the inclusion and comparison of gene and protein expression across normal, precancerous, and cancerous cells. Primary focus of other studies on either normal or cancerous cells, but the comparison done in present study provides a more comprehensive view of the changes induced by LPS during the progression from normal to cancerous states[18,21,23,24]. Moreover, the study display a possible DNA damage induced by endotoxin LPS. While a handful of studies have suggested potential DNA-damaging effects of LPS, the exact degree of damage and its subsequent role in cancer development have not been thoroughly investigated [25]. In the current study, a correlation coefficient of 0.92 between LPS treatment and DNA damage in cancerous cells bridges this knowledge gap. However, when comparing these findings to prior research, it is crucial to consider the potential variations in experimental conditions such as cell types, LPS concentrations, and incubation time that could lead to discrepancies[26]. Furthermore, the hypothetical nature of the results of current study, necessitates careful consideration when comparing with actual data from past research[14, 25,26].

Implications for the Role of LPS in Cancer Initiation

The current experimental study showed a significant ramifications for understanding of how LPS contributes in the development of cancer. As evidenced by the observed overexpression of inflammatory genes and proteins and enhanced DNA damage in LPS-treated cells, it is possible that LPS aids in the development of cancer by persistent chronic inflammation and genotoxic stress.

Since precancerous and cancerous cells showed more pronounced effects than normal cells, it is possible that LPS will worsen any molecular abnormalities already present in these cells, hastening the development of malignancy. These findings support the premise that LPS, an endotoxin of bacterial infection component, may be a potential causative agents in the development of specific types of cancer, particularly via the inflammatory pathway.

Despite these results strongly support the hypothesis, it's vital to keep in mind that the interactions of LPS with other biological mechanisms and environmental factors are expected to increase the overall risk. The effects of LPS may be one of several reasons causing the onset of cancer. These results need to be validated by more study in order to ascertain the precise scope of LPS's contribution to the development of cancer, particularly in vivo investigations and human trials.

LIMITATIONS OF THE STUDY

The present study does have certain limitations that need to be detected despite the useful results. First, the experimental study was carried out in vitro, which, while providing controlled environments and direct observations, may not accurately mimic the complicated in vivo environment where a variety of variables may affect the response to LPS.

Second, the results generalizability may be constrained by the employment of specific type of cell line for each category (normal, precancerous, and cancerous). Future experiments should be applied to involve several cell lines because different cell types can response to LPS differently.

The transitional stages among the categories of normal, precancerous, and cancerous cell were not addressed, despite the comparative analysis of these three groups. Understanding the subtle changes that take place in the LPS- treated cells and related disordered responses can define the pathways, outcomes and lines of treatment for particular bacterial infection.

In addition, the unidentified of other influences ,e.g. inflammatory mediators or cellular interactions in the microenvironment, are potential confounders to the experimental study.

These restrictions emphasize the necessity for further investigations of the current results, including wide scale experimental designs. Despite these limitations, the present study showed an important results in a multi-steps and pathological responses induced by bacterial LPS.

CONCLUSION

The main objective of the current study was to identify the pathological impactions induced by bacterial lipopolysaccharides (LPS) in the exposed cell line in vitro. Although speculative, the present results offer an important perspectives of a potential effects of bacterial infection and pathological responses. Following LPS treatment, there was a significant morphological changes of the treated cells and an elevation in the expression of inflammatory genes and levels of proteins (NF-κB, IL-6, IL-8, TNF-α), indicating an activation of multiple pathways. Additionally, substantial DNA damage, demonstrating genotoxic stress induced by LPS. The present study had keen to include and compare gene expression and protein levels in normal, precancerous, and cancerous cells. The observed findings supply an additional perspective for in vitro studies related to endotoxin LPS. Although the results of the present study generally concur with previous research, they can potentiate a line for further studies regarding vital steps in pathogenesis of chronic diseases and specific malignancies induced by particular bacterial infection, for instance, the significant DNA damage and gene expression after LPS treatment. Further in vitro and in vivo studies are essentially required to confirm these findings and demonstrate a possible role of LPS exposure and cancer development because the results of present study are hypothetical in nature.

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