

Microbial Infections in Immunodeficient Children in Qena University Hospital with Special Reference to DNA Damage in Peripheral Blood Leucocytes**Mona M.Mohamed^a, Mohammed B. EL-Mahdy^b, Ahmed EL-Abd^c, Mohammed A.El-Feky^d**^a Microbiology Department, Faculty of Medicine, South Valley University.^b Zoology Department, Faculty of Science, South Valley University.^c Pediatric Department, Faculty of Medicine, South Valley University.^d Microbiology Department, Faculty of Medicine, Assuit University.**Abstract**

Background: Immunodeficiency disorders involve malfunction of the immune system, resulting in infections that develop and recur more frequently, are more severe, and last longer than usual, Impair the immune system's ability to defend the body against foreign or abnormal cells that invade or attack it (such as bacteria, viruses, fungi, and cancer cells). As a result, unusual bacterial, viral, or fungal infections or lymphomas or other cancers may develop.

Patients and methods: A study to detect blood stream infections was done using 100 blood cultures obtained from 100 immunocompromised children.

Results and conclusions: The most commonly isolated are bacterial organism (40 %)the most common bacterial isolates are klebsiella species (17 | 40 ,42.5%). Estimation of DNA damage was done for 40 patients and 10 apparently healthy children as control subjects. The DNA damage was detected has took the smear shape on gel electrophoresis. This was found in (12|40, 30%), (P value < 0.001) with significant difference.

Keywords: Infections; DNA damage; Immunodeficiency; Qena.

Introduction:

An immunocompromised host is a patient who does not have the ability to respond normally to an infection due to an impaired or weakened immune system. This inability to fight infection can be caused by a number of conditions including illness and disease (eg, diabetes, HIV), malnutrition, and drugs.

According to new guidelines from the Infectious Diseases Society of America (IDSA), most immunocompromised patients should be vaccinated. The new guidelines are designed for health care professionals caring for patients with compromised immune systems due to HIV infection or AIDS, cancer, solid organ transplantation, stem cell transplantation, sickle cell disease or asplenia, congenital immune deficiencies, chronic inflammatory conditions, cochlear implants, or cerebrospinal fluid leaks (Barclay, 2014).

There are two types of immunodeficiency disorders:

- **Primary:** These disorders are usually present at birth and are usually hereditary. They typically become evident during infancy or childhood. There are more than 100 primary immunodeficiency disorders. All are relatively rare (Grammatikos and Tsokos, 2012).
- **Secondary:** These disorders generally develop later in life and often result from use of certain drugs or from another disorder, such as diabetes or human immunodeficiency virus (HIV) infection. They are more common than primary immunodeficiency disorders

Principles of infections of immunocompromised patients:

- Immunocompromised patient has alterations of innate defense mechanisms leading to an increased risk of severe infections.
- Infections are usually caused by pathogens with a low virulence.
- Course of infections due to virulent microorganisms is severe.
- Absence or alteration of immune response modifies clinical picture.

- Rapid diagnostics is important because infections are frequently fatal (Grammatikos and Tsokos, 2012).

Cellular DNA damage can be enhanced by exposure to various chemicals, environmental pollutants, steroids, hormones, and radiations. The increase in DNA damage has been reported to be associated with infectious diseases and dietary habits (Sly and Flacks, 2008).

The endogenous level of DNA damage in human peripheral blood leucocytes (PBL) has been extensively used as biomarker in studying the genotoxic effects associated with diseases, microbial infections, aging or exposure to the exogenous agents (Tsai et al., 2013).

The DNA integrity and or damage are central to the development of cancer and most human cancer is associated with DNA instability (EL-Sayed, 2005). In living cells, reactive oxygen species can lead to several types of DNA damage which may take several forms such as single and double stranded breakage (Thompson and Linoli, 2002) and lead to development of apoptosis (programmed cell death) (David et al., 2003). Necrosis is an accidental cell death resulting from severe and sudden thermal, physical, or chemical trauma (Wyllie, 1992). These cells typically swell and burst. They spill their contents all over their neighbors causing a potentially damaging inflammatory response (Alberts et al., 2002). Necrosis is accompanied by random DNA breakdown, with diffuse smear in agarose gels (Hassab EL-Nabi, 2004).

Patients and methods:**Patients:**

The present study is a hospital based descriptive study conducted from April 2016 to April 2017.

The study include immunodeficient children admitted to Pediatric department at QENA University hospitals and have infection

. They will be classifying into two groups:

Group 1 (Patient group) it included 100 immunodeficient children admitted to

Pediatric department at QENA University hospitals and have infection. clinical samples were collected under complete aseptic conditions according to sites of infection. 100 blood cultures collected during the study period were screened for bacterial and fungal infections and 100 blood sampel collected for DNA extraction to study DNA damage in peripheral blood leucocytes in immunodeficient children .

Group II (Control group): were include 10 apparently healthy children attending the outpatient clinic of the same hospital. Blood samples will collected to serve as a control.

Methods:

I- All samples will be identified by:

1- Staining and direct microscopic examination.

2- Culture on different types of culture media:

(simple media – enriched media – selective media)

- **Blood samples:** cultured on blood culture then make subculture on different types of media (blood agar – mannitol salt agar – macConkey media- chocolate agar- sabaraud dextrose agar).

N.B : fungal isolation will be done on sabaraud dextrose agar and hichrome media.

All subjects in the two groups were subjected to full history taking and physical examination

All sampels were cultured on blood culture for 48 houres then subcultured on blood agar ,mannitol salt agar , MacConkeys agar ,EMB media and Sabaraud dextrose agar & incubated for 24 to 48 hours at 37° c .

Klebsiella spp. colonies are similar to Enterobacter spp. Colonies but more mucoid so they can coalesce together with prolonged incubation (**Abbott, 2007**).

All lactose fermenter colonies on MacConkey agar were subcultured on EMB agar and plates were incubated at 35-37°C for 24h. According to: (**Bachoon et al., 2008**)

EMB agar plate inoculated with Klebsiellapneumoniae show good growth of pink or purple, dark-centered, mucoid colonies

(smaller than Enterobacter) indicating lactose fermentation and acid production..

EMB agar plate inoculated with Escherichia coli show good growth of dark blue-black colonies with metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment.

II-Identification of DNA damage in peripheral blood leucocytes using electrophoresistechnique:

DNA Extraction from Blood Sample by manual extraction :

- Nucleic acid extraction was based on salting out extraction method(**Aljanabi and Martinez, 1997**)whereas protein was precipitated by saturated solution of NaCl(**Hassab El-Nabi, 2004**)
- Lymphocytes from whole blood were separated by lysing the red blood cells (RBCs) using a hypotonic buffer (ammonium bicarbonate and ammonium chloride; Himedia) with minimal lysing effect on lymphocytes.
- Three volumes of RBC lysis buffer was added to blood sample and mixed by vortexing and inverting thoroughly for 5 min and centrifuged (Eppendorf 5415R) at 20,00 g for 10 min.
- The supernatant was mostly discarded, leaving behind ~1 ml to prevent loss of cells. To the pellet, 3 vol RBC lysis buffer was added, and vortexing, inverting, and centrifuging steps were repeated two to three times until a clear supernatant and a clean white pellet were obtained. After the final wash, the supernatant was discarded completely, and the pellet was resuspended in 500 µl PBS, followed by addition of 400 µl cell lysis buffer (10 mMTris-HCl, 10 mM EDTA, 50 mMNaCl, 10% SDS, pH 7.5) and 10 µl proteinase K (10 mg/ml stock; Himedia).
- The sample was vortexed to dissolve the pellet completely and incubated

for 2 h at 56°C in a water bath (CW-30G; Jeio Tech) for lysis.

- An equal volume of phenol (equilibrated with Tris, pH 8) was subsequently added to the tube and mixed well by inverting for 1 min. The tube was centrifuged at 10,000 g (at 4°C) for 10 min, and the aqueous upper layer was transferred to a fresh tube containing equal volumes (1:1) of phenol and chloroform:isoamyl alcohol (24:1). The tube was mixed by inverting for 1 min and centrifuged for 10 min at 10,000 g (at 4°C). The supernatant was then transferred to a fresh tube, and 10 µl of 10 mg/ml RNaseA (Fermentas, Thermo Scientific) was added.
- The sample was incubated at 37°C for 30 min before an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by inverting the tube for 1 min and centrifuging at 10,000 g (at 4°C) for 10 min.
- The supernatant was transferred to a fresh tube, and twice the volume of absolute alcohol (Merck) was added and inverted gently a few times and chilled at -20°C, followed by centrifugation at 10,000 g at (4°C) for 20 min. The supernatant was discarded, 250 µl 70% ethanol was added, and the pellet was tapped gently, followed by centrifugation at 10,000 rpm for 10 min and decanting the supernatant gently. The pellet was air-dried in a laminar air flow, and the dried pellet was resuspended in 50 µl nuclease-free water or 1× TE buffer and frozen at -20°C or -80°C for storage.

Materials used for agarose gel electrophoresis: (Lee et al., 2012).

TRIS Acetate EDTA Buffer (TAE)
(Electrophoresis Buffer)

Agarose gel (1.2%)

TAE 100 ml

Ethidium Bromide solution 0.5 Mg/ml

DNA Molecular Weight Marker

Detection:

Agarose Gel Electrophoresis for the Separation of DNA Fragments (Ferreira et al., 2011).

- 1- 1% electrophoresis grade agarose was prepared in electrophoresis buffer (TAE). The mixture was heated in a microwave (Samsung), for 1 minute to be sure that all agarose particles were completely melted. It was allowed to cool to 60°C, and then ethidium bromide (0.5% µg/ml) was added and mixed thoroughly.
- 2- The mixture was poured directly on the movable –casting apparatus. This was done after proper instillation of the desired comb in a position so that at least 1 cm gel layer was formed below the teeth of the comb. This was done to prevent the escape and migration of DNA below gel.
- 3- The gel was allowed to solidify at room temperature. then the tap was removed from the open ends of the gel platform and the gel comb was withdrawn.
- 4- The gel- casting platform containing the gel was placed in the electrophoresis tank. Sufficient electrophoresis buffer was added to cover the gel to a depth of about 1 mm (or just until the tops of the wells are submerged)
- 5- Ten µl (3 µl loading dye +7µl DNA) from each sample was loaded into each well using micropipette. Appropriate DNA molecular weight marker from 100-1000 bp was applied (7 µl DNA+3µl loading dye).
- 6- The leads were attached so that the DNA will migrate into the gel toward the positive lead. The samples were electrophoresed at 80 volts for 1 hour and monitoring of dye (bromophenol blue in the loading dye) was allowed to run 2/3 of the gel length before terminating the run.
- 7- The power supply was turned off when the dye from the loading buffer had migrated a

distance judged sufficient for the separation of the DNA fragments, the DNA was visualized by placing the gel on an UV Tran- illuminator.

- 8- The gel was transferred to the U.V. transilluminator to observe the damaged DNA on the gel according to its molecular weight comparing with the marker. The smear of damaged DNA were observed at 200 bp.
- 9- It was photographed by a digital camera and the image was analysed.

Statistical analysis:

- The study data will be statistically analyzed using the statistical package for social version program (spss program version 17.0. spss Inc., Chicago, Il. USA).
- Quantitative variable will be done using ANOVA, F test and Kruskal all is for testing association between categorized variable chi square and Monte Carlo will be used. Pearson's correlation test will be done to study possible correlation between quantities' variable.

Ethics consideration:

All patients will be included in the study after taking a written consent from the patients' parents after full explanation of the purpose, nature and risks of all procedures used according to the ethical committee of the Qena university hospitals.

Results:

In the current study we found that bacterial infection is the most common isolates. 40 % of collected sampels were bacterial isolates from blood.

The most commonly isolated organism were klebsiella species (17 | 40, 42.5%) followed by E.coli (9 |40 ,22.5%).

Estimation of DNA Damage was done for 40 patients who have blood stream infection and 10 apparently healthy children as control subjects.

The DNA damage was detected has took the smear shape on gel electrophoresis indicating double stranded DNAdamage .this was found in (12|40 , 30%) ,(P value < 0.001) with significant difference.

Table 1disease * DNA damage Cross tabulation

Disease	Dna damage		Total
	Negative	Positive	
acute hepatic failure	0	1	1
Bronchopneumonia	2	0	2
Diabetic	9	6	15
DIC	0	1	1
Malnutrition	2	0	2
Marasmus	0	1	1
nephrotic \$	8	1	9
Recurrent pneumonia	1	2	3
renal failure	1	0	1
Septicemia	4	0	4
Splenectomized	1	0	1
Control	10	0	10
Total	38	12	50

Table 1 Describe that diabetes is the most common immunodeficient disease between children and the highest percentage of DNA damage in diabetic patients 6\15 patient

Table 2 Infection *DNA damage Cross tabulation

Infection	Dna damage		Total
	Negative	Positive	
E.coli	8	4	12
Klebsiella	11	6	17
Pneumococci	1	0	1
Pseudomonas	1	0	1
staph aureus	7	2	9
Total	28	12	40
Control	10	0	10

Table 2 Describe that klebsiella and *E.coli* is the most common bacterial infections between immunodeficient children

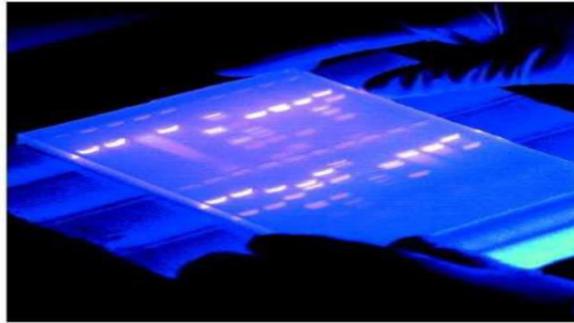


Figure.1. Gel electrophoresis showing smear shaped form of peripheral blood leucocytes indicate DNA damage double stranded in some children with bacterial infection.

Discussion:

In the current study we found that bacterial infection is the most common isolates . 40 % of collected samples were bacterial isolates from blood , this in agree with (Murray et al

In the current study we found that the most commonly isolated organism were klebsiella species (42.5%) followed by E.coli (22.5%) especially in diabetic patients.but Vollmer et al. (2008) found that invasive fungal infections caused by candida species and aspergillus species is the most common isolates this is due to his study was concentrated on fungal infection in immunodeficient patients and Vollmer et al (2008) used Real – Time PCR for early detection of fungal infection in immunodeficient children .

In the current study 30 % of immunocompromised children who have infection developed DNA damage appear as smear in gel electrophoresis indicating double stranded DNA damage this is in agree with Grivennikov et al. (2010) who found that Upon infection, bacterial cell components stimulate host pathogen recognition receptors, provoking chronic inflammation with a constant production of reactive oxygen species , reactive nitrogen intermediates, and cytokines by inflammatory cells such as macrophages and neutrophils. Chemical mediators of inflammation can damage proteins, lipids, metabolites, DNA, and RNA.

.,2005) who found that bacteria were the commonest organisms isolated from blood stream infections.

Bacteria that provoke chronic inflammation have been shown to promote carcinogenesis.

Also Polk and Peck (2010) found that chronic inflammation is presumed to be involved in 25% of all cancer cases. The best studied of these bacteria is Helicobacter pylori, which is associated with gastritis, peptic ulceration, gastric carcinoma, and mucosa associated lymphoid tissue lymphoma. Infection with H. pylori provokes DBSs, triggering a damage-signaling and repair response. Nevertheless, chronic infection with H. pylori also promotes down regulation of the two DNA repair mechanisms mismatch repair and base excision repair (Machado et al., 2010).

Focusing on the relation between bacteremia and possibility of presence of DNA damage it has been known that , cellular DNA damage took two forms , the first was the DNA laddering , indicating apoptosis (during apoptosis DNA nuclease cleave DNA at internucleosomal sites leading to production of DNA fragments of 200 bp and its multiples). The second form of DNA damage took " smear shape " on gel electrophoresis, indicating double strand breakage conferring fragments of extremely variable sizes (necrosis) (Hassab EL –Nabi, 2004).

Among the main finding in these study was the significant association between diabetes mellitus and DNA damage in peripheral blood leucocytes .this was clear in 6\15 diabetic children who have blood stream infection , this work came in agreement with(**Mohammed et al., 2005**) who found asmeas DNA damage in three diabetic mothers and in two of their infants. There was asignificant positive relation between bacteremia and peripheral blood leucocytes DNA damage.

In line with our finding, there was clinical evidence showing diabetes as astate predisposing to DNAdamage and apoptosis as was shown by **Mishra et al. (2005)** who reported that hyperglycemias evoked an intrinsic pathway of proapoptotic signaling in human mesangial cells cultured under high glucose concentration.

Conclusion

The methods used in these study is a simple, low coast and reliable method for detection of infection and DNA damage. A significant

Also **Nishikawa et al. (2000)** had shown that exposure of endothelial cells torelevant physiological glucose concentration lead to excessive flux of glucose through the cell membrane in an insulin independent manner via glucose transporter 1.this induces generation of reactive oxygen species , particulary super oxide which is the firstand key event in the activation of all other pathways involved in the pathogenesis of diabetic complications including the direct DNAdamage, protein and lipid damage.

Organized DNA damage is closely associated with altered immune function, development of autoimmunity and tumorigenesis. **El-sayed et al. (2005)** reported that an increase in DNA damage was associated with infectiouse diseases.

DNA damage present inimmunodeficient children with bacterial infections

Diabetic children who have infection are more reliable to develop DNA damage and cancers in the future.

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