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Original Research Paper

Human Beta Defensins-3 Gene Expression in Epithelial Cell and Neutrophils for Children with Leukemia before and after Treatment

Hanan S. Makii[1], Awatif H. Issa[2]* and Abdullah H. Al Saadoon[2]

[1]Basra Children Specialty Hospital, Basra Province, Iraq
[2]Biology Department, College of Science, University of Basrah, Basra, Iraq.

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Human β-defensin-3 (*HβD-3*) is the most interesting member of the antimicrobial peptide family. It has abroad spectrum of antimicrobial peptide activity against bacterial, fungal and some viral infections. *HβD-3* gene expression was detected in about 60 specimens from 15 patients before and after chemotherapy for each neutrophil and epithelial cell used for the study of *HβD3* gene expression. Control samples were 5 patients without chemotherapy suffering from different diseases such as oropharyngitis, tonsillitis, esophagitis and other inflammations. Acute Lymphoblastic Leukemia (ALL) was the most prevalent among patients with leukemia. *HβD-3* expression in epithelial cell (buccal mucosa) and neutrophils before chemotherapy and after chemotherapy (induction phase) was determined.

Keywords: HβD-3 expression, Molecular identification, Oral cavity, Leukemia patients.

INTRODUCTION

Defensins are highly spread in cells and tissues related with host defense against microbial agents. (Raj and Dentino, 2002). Defensins are considered as the largest antimicrobial peptide groups in mammals (Ganz, 2003; Lehrer, 2004; Lai and Gallo, 2009; Zhu and Gao, 2013). *HβD-3* consists of 45 amino acid residues, cationic peptide and the charged residues are distributed asymmetrically. They exist in most eukaryotic kingdoms referred to a common ancestor (Pazgiera *et al*., 2006; Clarke *et al.*, 2006 and Domingues *et al.*, 2014).

HβD-3 has a low sequence similarity among β-defensin class of peptides (Boniotto *et al*., 2003). This peptide is isolated and characterized from psoriatic scales then classified as an individual of beta-defensins family of peptide (Harder *et al*., 2005). *HβD-3* induced expressions are found in different sites of the body, where high levels are observed in the trachea, tonsil, tongue, skin and low expression level in salivary gland, stomach, uterus, pharynx, and other sites by the use of real time-polymerase chain reaction (Dunsche *et al*., 2002).

Additionally defensins are representing endogenous antibiotic agents that give chemically protection for the mucosal surface, and their relative decrease may cause microorganisms adherence to the epithelial surface, the slow invasion of microbial infection into tissues, and secondary inflammation for mucosal cells (Fellermann *et al*., 2006).

Several studies have been conducted on expression of human beta-defensin-3 including the study by Harder *et al.,* (2001) in Kiel city, Germany in which the isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic was carried out. A study by Beverly *et al.,* (2005) conducted in the USA has shown the expression and function of antimicrobial Peptides in innate immune responses in the oral cavity in addition to regulation of β-defensins which has an important role in oral epithelial innate immunity.

In another study by Dhople *et al.,* (2006) in the University of Michigan, USA, the role of *hβD-3* as an antimicrobial peptide with multiple biological functions including immune modulatory function, tissue reorganization and wound healing was shown. *Silva et al*., (2014) in their study conducted in Portugal and focus of intense research toward the defensins, as other AMPs, have shown to be an effective alternative to the current antimycotic therapies, demonstrating potential as novel therapeutic agents or drug leads. While the study by Lee RM *et al*., (2015) conducted in the United Kingdom showed that defensin copy number variation is an important contributor to maximizing the host innate and adaptive response.

The cancer patients have low host defense immunity, when immune response systems are suppressed resulting from administered chemotherapy such as individuals with leukemia which can be defined as cancer of the early blood-forming cells. And the study revealed that the leukemia was more

*Corresponding Author: Awatif H.Issa. Basra Children Specialty Hospital, Basra Province, Iraq. Email: awatifhi@gmail.com

frequent in the age group 2-4 Valera *et al.,* (2015). Acute lymphoblastic leukemia (ALL), a malignant disorder of lymphoid progenitor cells, is the most common type of leukemia among children, accounting for 75% of all childhood leukemia (Yeoh *et al., 2013;* Wang *et al.,* 2014 and Girish Babu *et al.,* 2016).

MATERIAL AND METHODS

Patients and Specimens

This study was conducted at Basra children specialty hospital, Basra province, Iraq from August 2016 to June 2017. The specimens were collected from children before and after (4-6 weeks) receiving chemotherapy. About 60 specimens from 15 patients before and after chemotherapy for each neutrophil and epithelial cell were used for study of *HβD-3* gene expression. Control samples were 5 patients without chemotherapy (2 males and 3 females) suffering from different diseases such as oropharyngitis, tonsillitis, esophagitis and other inflammations. In addition, both groups were examined for signs and symptoms of oral infections.

Collection of Specimens

In this study two types of specimens were collected: (1) whole blood collected in EDTA tube was used for RNA extraction. (2) swab of buccal mucosa epithelial cell was used for RNA extraction.

Type of chemotherapy treatment

According to Basra Children specialty Hospital, Oncology Center, the therapeutic protocol used in the treatment of patients with leukemia, which includes a number of chemical treatments given to the patient with a certain dose by age and weight for each patient and these treatments include:

Vincristen 1.5 mg /m^2, Daunorubicine 30 mg/m^2, L-asparginase 5.000 U/ m^2
Cyclophosphamide 1.000 mg/m^2, Mesna 400 mg/ m^2,
Cytarabine 75 mg/m^2
6-Mercaptopurine 60mg/m^2, Prednisone 60mg/ m^2 and
Methatroxate 6 - 12 mg / m^2, according to age from less than one year into more than 3 years .

Molecular methods

Total RNA Extraction: After collecting the specimens (epithelial cell and whole blood), RNA extraction was initiated immediately because of the RNA rapid degradation via RNase enzyme. In this study, the commercial manual kit called AccuZol from Bioneer Company was used. This kit was used for total RNA isolation according to manufacturer's protocol. The RNA preparation was dissolved in 50 - 100 µl RNase free water depending on the size of pellet. And before the solution was stored at -70 °C the concentration of RNA samples were measured by Nanodrop device (Optizen popNano Bio).

RT-PCR: Total RNA was converted into cDNA by the use of Accupower Rocket Script RT PreMix kit from Bioneer company as follows: Template RNA, DEPC water and oligo dT15 primer were thawed before use. And 5µl of total RNA, 2 µl of oligo dT15 and 13 µl of DEPC water were added into the Accupower Rocket Script RT PreMix tubes and the reaction was performed under the following conditions. Primer annealing at 30 OC for 10 min, cDNA synthesis at 42 °C for 45min and heat inactivation at 95 OC for 5 min. The synthesized cDNA was then amplified by RT-q PCR using specific sense F 5-AGCCTAGCAGCTATGAGGATG-3 and antisense primers R 5-CTTCGGCAGCATTTTGCGCCA-3 for the genes of interest (HβD3 gene) with a housekeeping gene (β-actin) sense F5-CTCCTTAATGTCACGCAGGATTTC-3 and antisense R 5-GTGGGGCGCCCCAGGCACCA-3 (Schroeder, J. et al., 2001). Which were provided by Alpha DNA Company. Samples were loaded into Green Start q PCR PreMix tube that contains SYBER Green I dye which is used to detect PCR product in real-time monitoring by using the protocol Exicycler 96 (Bioneer.com), for an initial denaturation at 95.00 °C for 10 min, followed by 45 cycles, each cycle consisting of 95.00 °C for 10 sec, 60.00 °C for sec, and 72.00 °C for 10 sec. At the end of each run, melting curve profiles were produced.

Statistical Analysis: Statistical analysis of data was carried out by using the t-test sample (paired samples test and paired samples correlation) with differences at $P<0.05$ which is considered to be statistically significant. This calculation was carried out according to the Statistical Package for Social Science (SPSS version 20) and the least significant difference at a level less than (0.05).

RESULTS AND DISCUSSION

This study was conducted on about 15 children patients with leukemia before and after chemotherapy for each neutrophils and epithelial cells used for *HβD-3* study, and the range of their age between (2-16 years). The study revealed that the leukemia was more frequent in the age group 2-4 (40%). While the age group 14-16 (6%) was the less frequent (see Table1).

The frequency of leukemia particular type Acute Lymphoblastic Leukemia (ALL) in children patients was more than leukemia type Acute Myeloid Leukemia (AML) that agrees with (Yeoh *et al., 2013;* Wang *et al.,* 2014 and Girish Babu *et al.,* 2016). The results of the current study showed that most of the children patients in both cases before and after chemotherapy were suffering from neutropenia that increases invading opportunistic infections in immune suppression patients.

Recent study had shown an increase of immunocompromised patients that result from increased levels of chemotherapy, radiation, high doses of corticosteroids, individuals with diabetes mellitus and HIV which inhibit the immune system (Teoh and Pavelka 2016). Expression of *hβD-3* in neutrophils of children patients before chemotherapy was (0.865195) with significant difference ($P< 0.05$) and this value decreased to (0.087716) after chemotherapy with no significant difference ($P> 0.05$) and there was highly correlation between the expression of *hβD-3* in neutrophils of children patients before and after chemotherapy because most children patients with leukemia in this study were suffering from neutropenia before chemotherapy and increased number patients with neutropenia after chemotherapy, when the number of absolute neutrophils count was less than 500 cell /mm^3, this results was consistent with Paus *et al.*, (2013) Figure (1).

The expression of *hβD-3* gene in epithelial cells of children patients before chemotherapy was (0.382182). Even with low level of *hβD-3* expression, the bacteria and yeasts were isolated from the oral cavity (Alsaadoon *et al*., 2017 and Makii 2017).

Table 1: Description of the *HβD-3* study pattern for patients at Basra children specialty hospital

Age classes	Patients with leukemia from n=15 patients , about 60 specimens before and after chemotherapy for each neutrophils and epithelial cells used for *HβD-3* study		Patient with inflammation As control n= 5 used for *HβD-3* study	
	No.	%	No.	%
2-4	6	40%	4	80%
5-7	4	27%		
8-10	4	27%	1	20%
11-13	/	0%		
14-16	1	6%		
Mean ±SD	3± 2.449		2.5±2.12132	
Range	2- 16		2- 8	

Sex	Type of leukemia				Total
	ALL	%	AML	%	
Male	10	67 %	2	13%	80%
Female	3	20 %	0	/	20%
Total	13	87%	2	13%	100%

n=15 Patient with leukemia used for HβD-3 study, n =5 patient without leukemia (with inflammation) was used as control for *HβD-3* study, **ALL:** Acute Lymphoblastic Leukemia, **AML:** Acute Myeloid Leukemia.

Table 2: Statistical analysis of HβD-3 gene expression for children calculated twice before and after chemotherapy.

No.	Type sample	Correlation coefficient	P value
1	HβD-3 expression for Epithelial cell	1	0.5
	HβD-3 expression for Neutrophills	1	0.5

P > 0.05 No significant difference

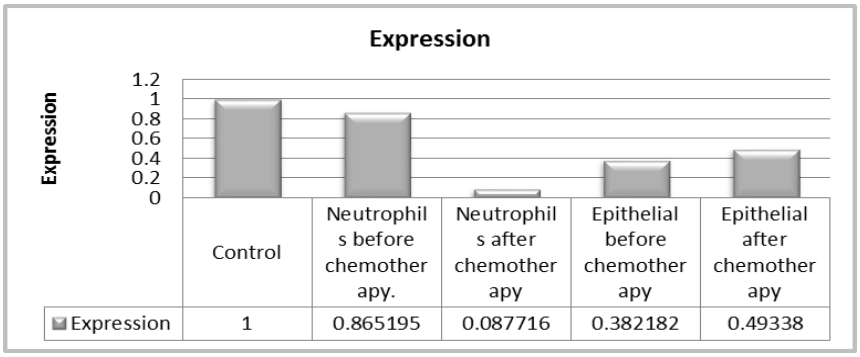

Figure 1: Relative expression of *HβD-3* gene before and after chemotherapy.

P < 0.05 Significant difference for *HβD-3*expression for neutrophils before chemotherapy,
P > 0.05 No significant difference for *HβD-3* expression for neutrophils after chemotherapy, Epithelial cells before and after chemotherapy.

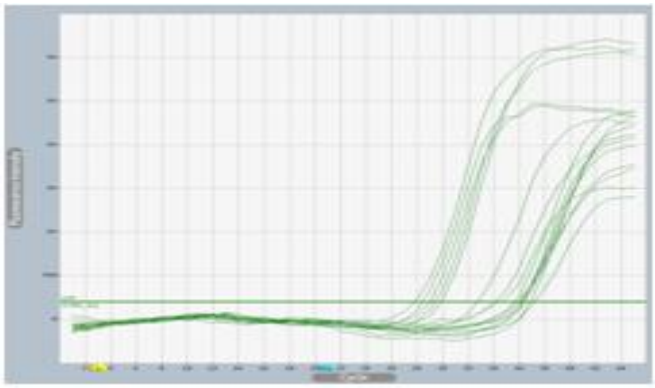

Figure 2: Quantification of mRNA level by RT-PCR for HβD- 3

The expression of *hβD- 3* gene in epithelial cells was increased after patients received chemotherapy up to (0.457338), and this simple shift in amount of expression *hβD- 3* gene between before and after chemotherapy may be caused from dose of chemotherapy during induction phase (4 - 6 weeks) this results is consistent with Paus *et al.*, (2013) which exhibited detrimental effect for chemotherapy on epithelial layer.

From another side this increased of *hβD-3* gene expression in the epithelial cell after chemotherapy was accompanied by an increase in *Candida species* practically *Candida albicanse* in the oral cavity. So this result is consistent with Dhople *et al.*, (2006) that state increase in mRNA levels of *hβD-3* observed when oral epithelial cells were challenged with *C. albicans*, with high correlation between epithelial cells expression before and after chemotherapy with no significant difference (P> 0.05) (see Table 2).

CONCLUSION

The current study of children with leukemia at Basra children specialty hospital ,showed that there was agenetic expression for *hBD-3* in epithelial cell (buccal mucosa) in the oral cavity and genetic expression for *hBD-3* in neutrophils before and after chemotherapy were less than expression for control sample patient (epithelial cell and neutrophils) before and after chemotherapy. Finally this study demonstrates that there is a relationship between genetic expression of *hBD-3* in epithelial cell and neutrophils.

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REFERENCES

Al-saadoon HA, Issa HA, Makii SH (2017). First recovery of Candida africana from oral cavity of children with leukemia in Basrah, southern Iraq (unpublished).

Beverly AD, Fredericks L P (2005) .Antimicrobial Peptides in the Oral Environment: Expression and Function in Health and Disease. Curr Issues Mol Biol. 2005 Jul; 7(2): 119–133.

Boniotto M, Antcheva N, Zelezetsky I, ossi A, Palumbo V, Verga Falzacappa MV, Sgubin S, Braida L, Amoroso A, Crovella S(2003). A study of host defence peptide β-defensin 3 in primates, Biochem. J. 374 707–714.

Clarke DJ, Campopiano DJ (2006). Structural and functional studies of defensin-inspired peptides. Biochem Soc Transac. (34): 251-256.

Dhople V, Krukemeyer A, Ramamoorthy A (2006). The human beta-defensin-3, an antibacterial peptide with multiple biological functions. Biochimica et Biophysica Acta 1758 :1499–1512.

Domingues MM, Silva PM, Franquelim HG, Carvalho FA, Castanho MA RB, Santos NC (2014). Antimicrobial protein rBPI-induced surfacechanges on Gram-negative and Gram-positive bacteria .Nanomedicine: Nanotechnology, Biology and Medicine 10 (3): 543-551.

Dunsche A, Acil Y, Dommisch H, Siebert R, Schroder JM, Jepsen S (2002). The novel human beta-defensin-3 is widely expressed in oral tissues, Eur. J. Oral Sci. 110: 121–124.

Fellermann K, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL (2006).A chromosome 8 genecluster polymorphism with low human beta-defensin gene copy number predisposes to Crohn disease of the colon. Am J Hum Gene.79(3):439-48.

Ganz T (2003). Defensins antimicrobial peptides of innate immunity Nature Reviews Immunoloi.3(9): 710-720.

Gao B, Zhu S (2013).Alteration of the mode of antibacterial action of a defensin by the amino-terminal loop substitution. Biochem Biophys. Res. Commun. 426: 630–635.

Girish Babu KL, Mathew J, Doddamani GM, Narasimhaiah JK, Naik LR(2016). Oral health of children with acute lymphoblastic leukemia. A review. J Orofac Sci .8.3-11.

Harder J, Bartels J, Christophers E, Schroder EJ-M (2001). Isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic. J. Biol. Chem.(276) :5707–5713.

Harder J, Schroder JM (2005). Psoriatic scales. a promising source for theisolation of human skin-derived antimicrobial proteins, J. Leukocyte Biol. 77 476–486.

Lai Y, Gallo RL (2009). AMPed up immunity: How antimicrobial peptides have multiple roles in immune defense. Trends Immunol.. 30:131–141.

Lee RM, Barbara O (2015). An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses.fro.J.immunol.6.

Lehrer RI, (2004). Primate defensins. Nat. Rev. Microbiol. 2: 727–738.

Makii SH, (2017). Expression of HβD-3 gene and associated with microbial infection for oral cavity in children with leukemia at Basra children specialty hospital. A thesis from College of Science-University of Basrah.

Pazgiera M, Hooverb DM, Yangc D, Lud W, Lubkowskia J. Human β-defensins (2006). Cell Mol Life Sci. 23:1294 -1313 .

Raj PA, Dentino AR(2002). Current status of defensins and their role in innate and adaptive immunity. FEMS Microbiol Lett; 206:9-18.

Schroeder J, Harder J, Bartels J, Enno C (2001). Isolation and Characterization of Human b-Defensin-3, a Novel Human Inducible Peptide Antibiotic. J Biological Chemistry. 276(8). 23, pp. 5707–5713.

Silva MP, Santos CN, Gonçalves S (2014). Defensins: antifungal lessons from eukaryotes. Frontiers J. Microbiol., 5.
Teoh F, Pavelka N (2016). How chemotherapy increases the risk of systemic candidiasis in cancer patients current paradigm and future directions. Pathogens. 5(6): 1-16.
Valera MC, Noirrit-Esclassan E, Pasquet M, et al (2015). Oral complications and dental care in children with acute lymphoblastic leukaemia. J Oral Pathol Med, 44, 483-9.
Wang Y, Xue J, Zhou X, You M, Du Q, Yang X (2014). Oral Microbiota Distinguishes Acute Lymphoblastic Leukemia Pediatric Hosts from Healthy Populations. PLoS ONE 9(7): e102116/10.1371/j. pone.0102116.
Yeoh AE, Tan D, Li CK et al., (2013). Management of adult and paediatric acute lymphoblastic leukaemia in Asia: resource stratified guidelines from the Asian Oncology Summit. Lancet Oncol. 14: 508-23.