The Role of Secretary Immunity in Diagnosis of Bacterial Lactational Mastitis in Women

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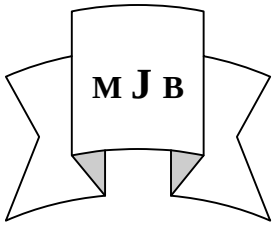

Abstract

Women lactational mastitis is an acute neutrophilic infection .The infections were of single , dimicrobic and stealth pathogen etiology . Gram positive cocci and gram negative rods were isolated from 50 lactional mastitis women but not from the 20 control women . Heat treated whole cell antigen for gram negative rod and benzal konium chloride for gram positive cocci were prepared . Mucosal immunoglobulins were separated partially ,characterized and titrated by standared tube agglutination .The base line titers ranged 2-4 .The clinical titres ;however ,were ranging 8-128,in single infection Antigenic competition were noted in two cases of mixed infection. The correlation between concentration of MMIg and titres was of simple linear type .MMIg can be used as diagnostic probe for mammary lactational mastitis in women .

الخلاصة

اتصف خمج الغدة اللبنية الرضاعي في النساء بأنه حاد مع ارتشاح العدلات في الحليب . كانت صورة الخمج احادي وثنائي ومتخفي المسبب . ووجد في هذه الخمجات بكتريا من كل من سلبية الغرام عصوية الشكل وايجابية الغرام مكورة الشكل . حضر مستضد الخلية الكامل المقتول بالحرارة في حالة سلبية الغرام والمعامل بمادة كلوريد البنزلكونيوم في حالة ايجابية الغرام وجرى فصل الكلوبيولين المناعي الافرازي من الحليب والتعرف الجزئي عليه ثم استخدم في الاستشعار عن الخمج من خلال اختبار تلازن الانبوبة القياسي بين البتكرينات المحضرة والكلوبين المناعي اللبني المفصول .

وقد لوحظ التنافس المستضدي بين حالتين ثنائية المسبب . مثل العيار التلازني 2- 4 عيار خط الشروع وكان العيار السريري يتراوح بين 8 و 128 . هناك علاقة خطية بسيطة بين تركيز الكلوبين المناعي اللبني وبين عيار الضد المتخصص مما وضح من نتائج يتبين ان الكلوبين المناعي اللبني ممكن الاستخدام بوصفه واسم مقبول للتحري عن خمج الغدة اللبنية الرضاعي في النساء .

Introduction

Mammary gland (MG) is a mucosal compartment of the common mucosal immune system (1).It is regarded as an effector sites that are responsible for production of secretory immunoglobulin (S-IgA)during antigenic stimulation of inductive sites ,such as gut associated lymphoid tissue ,nasopharynex and skin (2).During lactating mastitis mammary mucosal surface stimulated by the associated causal (3) to produce increasing amount of S-IgA (4-5).The aim of the present work is the determination of bacterial mastitis causals and the a companied inflammatory cells as well as separation of S-IgA with an attempt to use it as an infection probe .

Materials & Methods

From 60 women with lactating mastitis and from 20 normal lactating women as control milk samples were collected from December 2003 two April 2004 in Breast Disease Referral Center, Babylon Obstetric and Gynecology Hospital Hilla. Sample collection was under the sterile conditions and interview of an obstetrician in AFMA disposable tubes(6) Direct quadrate streak milk culture was on blood agar and MacConky agar, and indirect through pretreatment with broth media then plated on blood agar and MacConky agar. Direct defatinated milk fixed and stained with giemza for leucocyte differential count (7). The separation of S-IgA was done by Polyethylene (PEG) 6000, 6% (8) ,concentration and 2-ME effect from these milk sample were done as in(8,9). Growth from direct and indirect culture was identified using classical and API-20 minisystem (10) Whole cell antigens from gram positive bacteria by benzal chonium chloride 0.005% of 5IU density (WHO Geneva) as in MacCoy and Kennedy (11)was followed . While gram negative whole cell antigen was made as heat killed bacterin (12),with 5IU density. Direct slid agglutination and standard tube agglutination of mucosal IgG of test antigens (13) was performed .

Results

1-Mastitis ;

The dominance of mastitis in lactating women was 15-45 years age group (40:60,66.77%). Right breast was higher 32:60 ,53%,than left breast which was 28:60 ,47%. The inflammatory response was: 44.2, 31.1, 23.1, 1.3, and 0.4% for neutrophile,lymphocyte ,monocyte eosinophile and basophile respectively . The infection was in three

forms ,as single ,mixed and stealth. The microbial profile for single etiology was *Staphylococcus.aurus* *16:60(26.6%),Pseudomonas.aeruginosa* 13:60 (21%) ,*Klebsiella.pneumonie* 10:60 (16%) ,*Staphylococcus.epidermidis* 9:60(15%) , *Escherichia.coli* 2:60 (3.3%) , *Steptococcus.viridans* 1:60 (1.6%). Mixed infection was reported in rate of 4:60 (6.5%) as well as stealth 4:60 (6.5%) .

2- Mammary mucosal immune responses

Single mammary mucosal mastitis increases both mucosal immunoglobulin concentration and the bacteria specific mucosal antibody titres . The concentration of MMIg was ranging from 0.3-1.43 g/l and the specific antibody titres were ranging from 8-128 . The regression analysis between concentration and titres were of simple linear equation and the correlation coefficient was ranging between r = 0.7 and 0.8 , (Table-1) . In dimicrobic lactation mastitis the mucosal Ig concentration were ranging between 0.3-104g/l . 2-ME treatment did not reduce concentration or titre . Bacteria specific mucosal antibodies titres were ranging between 32 and 16 ; 128 and 16 ; 4 and 64 and 64 and 32 . Two cases were with antigenic competition and two without . (Table-2) Populational mammary mucosal responses of patients is depicted in table 3 and fig. 1

Discussion

1-Infection:

The source of infection could be exogenous, skin microflora ,cracks of nipple and baby microflora suckling .It can be of endogenous route from systemic infection or hidden locie in other body sites (14).At both cases ,however ,causals should evade the local mammary defenses .If succeeded it will produce through virulence quorum sensing signal to initiate local lesion ,mastitis (15,16).Milk stasis in breast may also causes mastits (14).The dominance of mastitis was in the age a range of 25-34 year is in agreement with Jonsson and Pulkinen 1994 (17).The profile of bacteria associated with mastitis was in agreement with Shnawa *et al* 2005 (18).Since it consist of single ,dimicrobic and stealth pathogen (18,19) .

2- Mammary mucosal immune responses

Bacteria in mucosal surfaces are taken up by macrophages ,denderitic cells and or langerhans cell processing phagocytic events to peptides polysaccride and or simple or conjugated lipid compounds . Such macromolecules are either conjugated with MHC II or MHC I molecules and take up to all surface (antigen processing and presenting) then presented to either Th2 which in turn

activate B-cells to grow up ,expand and become plasma cell producing mucosal antibodies at effector diffuse lymphoid tissues like mammary gland (20).The mammary mucosal antibodies base line titre in normal subject milk was 2-4 and 8 – 128 for patients .The infection of mammary gland induce , comparable mucosal antibodies for one causal in single or for both causals in dimicrobic infection that initiate elevated immune responses at mucosal surfaces may have an immunodominant epitope that give such rise in mucosal antibody titres which can be of T dependent or T independent types (20,22,23,24) one case was with reduced one fold in titre table 1 due to 2ME treatment which transudate of serum fraction (21). Thus on conclusion one may sum up the following

1. Lactation mastits is acute mostly neutrophilic .
2. The infection form are single , dimicrobic and stealth .
3. Mammary mucosal immunoglobulin is separated by PEG (6000 M.W) ,2-ME resistant with specific ab activity was 8-128 . The MMIg concentration and titre have shown linear simple regression equation with high correlation coefficient

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Table 1 Mammary mucoal immune response in monomicrobic lactation mastitis and normal subject .

Mastitis associated pathogen	No 50**	Age	MIg concentration Mean , median range	MIg titre Mean , median range	MIg Concentration ,titre correlation
Gram positive					
S.aureus	16	21-41	0.8 0.9 0.3-1.7	57 68 8-128	Y=-1.4+72.55x r=0.8
S.epdermidis	9	18-37	0.82 1.03 0.6-1.7	71 80 32-128	Y=-4.8+92.6x r=0.778
Gram negative					
P.auroginosa	13	24-40	0.75 0.78 0.3-1.4	36 72 16-128	Y=-6.462+56.6x r=0.7
K.pneumoniae	10	25-35	0.84 0.865 0.5-1.43	57.6 68 8-128	Y=-22.19+94.1x r=0.73
E.coli	2	27-30	1.063 1.063 0.7-1043	80 80 32-128	
Normal milk *S.epedermidis* 2:20	20		0.406 0.575 0.05-1.1	3 3 2-4	

*One case redused to one tube in titre due to 2-ME effect

** Total Number of patients .

Table 2 mammary mucosal immune response in dimicrobic mastitis.

Sequence	Age	Affeced breast	Associated Pathogen	MMIg Concenation , titre	Titre with 2-ME
1*	33	Right	*S.aureus & K.pneumoniae*	0.5 32&16NAGC	32&16 NAGC
2	30	Right	*S.epidermidis & P.aeroginosa*	0.3 128&16AGC	128&16AGC/no red
3	45	Left	*S.epidermidis & P.aeruginosa*	0.34 4&64 AGC	4&64 AGC/no Red
4	34	Left	*Actinomyces & S.aureus*	1.4 64&32NAGC	64&32NAGC/no Red

AGC:Antigic competition

NAGC:No antigenic competition

No red :2-ME no reduction effect

* cases of mixed infection are out of 50.

Table 3 Populational mammary mucosal response among patients with lactional mastitis.

Microorganism	Titer				
	8	16	32	64	128
S.aureu	1	3	5	2	3
S.epidermidis	-	-	4	2	3
P.aeruginosa	-	5	5	1	1
K.pneumoniae	4	-	1	2	3
E.coli	-	-	1	-	1
Sum	5	8	16	7	11

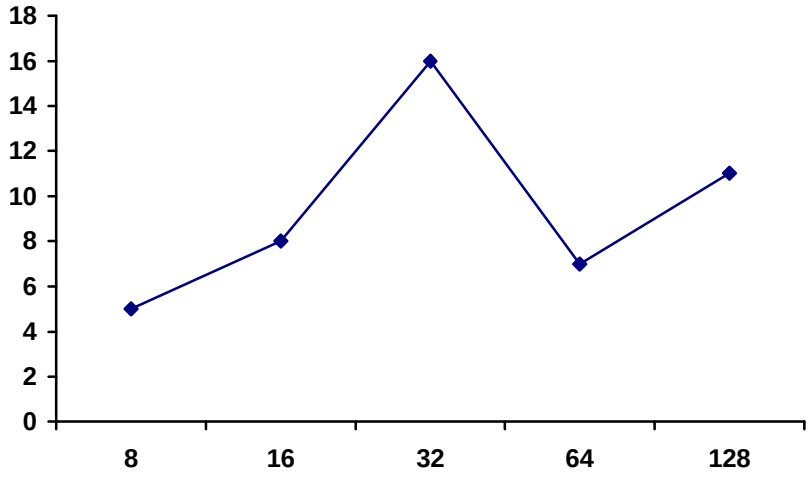

Figure 1 explain frequency of the titers within the cases.