Evaluation of immunoglobulines versus natural salivary defense in controlling recurrent herpes simplex lesions

Ihsan A. Kumail B.D.S. M.Sc (1)
Rajaa Al-Jobori B.D.S. M.Sc Ph.D (2)

ABSTRACT

Background: There has been no precise correlative study for evaluating the role of immunoglobulines versus salivary natural defense in controlling recurrent oro-labial herpes simplex infection. The present study aimed to evaluate the efficiency of immunoglobulines versus natural salivary defense in preventing recurrent lesions.

Method: This study was conducted on 88 healthy dental students in oral diagnosis clinic of Dentistry College/ Mustansirya University from October 2007 to December 2007. Evaluation of defense components was done by identifying and measuring the level of serum anti-HSV1 IgG, salivary secretory IgA and salivary hypothiocyanite in seropositive subjects with and without recurrent oro-labial herpetic lesions.

Results: It was found that the level of serum anti-HSV1 IgG was high in seropositive subjects with recurrent lesions and low in seropositive subjects without recurrence, while the level of salivary hypothiocyanite anion was markedly higher in seropositive subjects without recurrence than seropositive subjects with recurrence.

Conclusion: The natural salivary defense factor – Hypothiocyanite- was able to control recurrent lesions of HSV 1 when its concentration is ≥ 90 µM, while immunoglobulines are not efficient in controlling recurrence of the lesion.

Key words: immunoglobulines, natural defense, recurrent herpes lesion. (J Bagh Coll Dentistry 2009; 21(4): 63-69).

INTRODUCTION

The HSV1 life cycle has been well-characterized in humans. After primary infection of oro-facial mucosa or skin, the virus resides in a latent state in the neurons of the sensory ganglion of the affected region [1]. Various stimuli may re-activate HSV in latently infected neurons. Following re-activation, the virus migrates via sensory nerves to the epithelium, where it replicates and, under favorable circumstances, causes visible lesions, also known as recurrent HSV lesions [2].

After primary oro-labial herpes simplex infection, serum anti-HSV1 IgG will be formed (usually antibodies against viral surface glycoproteins) leads to neutralization of the newly attacking virus [3], as well as the reactivating latent virus [4], but how the acquired immune response protects against disseminated viral disease on one hand and yet is not completely effective in preventing recurrent viral infection is to be explained [5].

The role of saliva in the control of oral herpes simplex virus (HSV1) infection is controversial, since HSV can be isolated from saliva of asymptomatic HSV seropositive individuals [6, 7]. Furthermore, the use of more sensitive methods, such as detection of HSV nucleic acid in saliva by PCR, has shown that HSV shedding is actually more frequent than was previously believed [7]. Given the high frequency of HSV shedding in the absence of apparent disease, it seems likely that HSV1 may become inactivated to a certain level in the oral environment [8].

(1) PhD student, department of oral diagnosis, university of Baghdad.
(2) Professor, department of oral diagnosis, university of Baghdad.

An antimicrobial system consisting of peroxidase enzyme(s), hydrogen peroxide (H_2O_2), and thiocyanate ion (SCN-) has been detected in saliva [9], milk and perhaps also tears [10]. This system may contribute to the antimicrobial activity of exocrine secretions [11].

The antimicrobial activity of the peroxidase system is due to that peroxidase catalyzes the oxidation of SCN- either directly to hypothiocyanite ion (OSCN-) at neutral pH [18], or to thiocyanogen (SCN)2 at acidic pH [12, 13], which hydrolyzes rapidly to yield hypothiocyanous acid (HOSCN) or hypothiocyanite OSCN- [14].

The antimicrobial agent which accumulates at acidic pH is HOSCN which is in equilibrium state with OSCN- [14].

$$H_2O_2 + 2\ SCN^- + 2\ H^+ \longrightarrow (SCN)2 + 2\ H20$$

$$(SCN)2 + H20 \longrightarrow HOSCN + SCN^- + H^+$$

$$HOSCN \rightleftharpoons H^+ + OSCN^-$$

The antimicrobial agent which accumulates at neutral pH is OSCN- [9], which is consistent with the net reaction:

$$H_2O_2 + SCN \longrightarrow OSCN^- + H20$$

Entry of HSV into the host cell involves interactions of several glycoproteins (gB, gC, gD and gH) on the surface of the enveloped virus, with receptors (heparan sulfate HS and herpes virus entry mediator receptor HVEM) on the surface of the host cell [15]. The envelope covering the virus particle, when bound to those receptors will fuse with the host cell membrane and create

an opening, or pore, through which the virus enters the host cell [16].

More than 60% of population all over the world are HSV 1 seropositive [17], some are experiencing recurrent oro-labial lesions, while others are completely asymptomatic, why?, what are the factor(s) that may prevent reactivation of the latent virus, this is to be explained.

SUBJECTS AND METHODS

Sample collection

Subjects enrolled in this study with informed consent were 3rd, 4th and 5th year students of the Dentistry College/ Mustansirya University (mean age 22.5 years), they were clinically healthy and have not any systemic disease, they had been asked whether they had ever experienced recurrent labial or genital herpes and the frequency of recurrence, all informations were recorded carefully for each subject.

1- Immunoassays

All processing of immunoassays were done in the Directorate of Teaching Laboratories of the Medical City under supervision of Dr Nahla G. Abdulmajeed (specialist of immunity).

A- Detection and quantification of serum anti-HSV-1 IgG

This was done by using Herpes Simplex (HSV 1 IgG) kit Catalog no. 1A-401 from United Biotech Inc. (UBI MAGIWEL) U.S.A.

Five ml venous blood was collected from each subject at morning into labeled plastic test tubes, clotting was allowed and tubes were immediately taken to Directorate of Teaching Laboratories where sera were separated at the same day by centrifuging at 1500 rpm for 5 minutes.

Sera were collected into apendroff tubes and immediately assayed for identification and quantification of serum anti-HSV1 IgG according to leaflet of the kit.

Readings of results were done by using micro-well reader (type Sanofi Diagnostics Pasteur PR 2100 reader, ser. No.1CX03734 USA)

According to detection of anti-HSV-1 IgG in sera, subjects had been grouped into:

Group A: Seropositive with recurrent herpes labialis (RHL)

Group B: Seropositive without RHL

Group C: Seronagative

The level of anti-HSV-1 IgG was recorded for each subject in the two seropositive groups.

Collection and treatment of saliva

Collection of unstimulated saliva by drooling had been carried out in surgical clinic of Dentistry College/ Mustansirya University between 8-10 a.m. Subjects were asked to refrain from eating, drinking, chewing and smoking one hour prior to donation of saliva.

B- Quantification of salivary S-IgA

One ml of saliva was collected into labeled test tube, salivary flow rate was measured and recorded for each, all samples were taken to Directorate of Teaching Laboratories of the Medical City, frozen to precipitate mucin, thawed at the day of assay and centrifuged at 3000 rpm for 15 minutes (as directed by the leaflet of the kit).

This assay was done by using salivary secretary IgA indirect enzyme immunoassay kit (Catalog No. 1-1602, 96-Well Kit from Salimetrics USA).

Results were recorded according to groups.

2- Chemical assay

All chemical and biochemical procedures had been carried on in biochemistry laboratory of Dentistry College / Mustansirya University under supervision of specialist chemist, the remaining 4 ml of each sample of saliva were inserted into ice bag and immediately analyzed for concentration of hypothiocyanite OSCN-.

Hypothiocyanite was assayed by reducing 5,5' dithiobis-2 nitrobenzoic acid (DTNB or Nbs2) with 2- mercaptoethanol (2-ME) to produce the yellow colored anion 5-thio-2-nitrobenzoic acid (TNB or Nbs) [18, 19], then by mixing equal sizes of saliva and mixture, the hypothiocyanite present in saliva will reoxidize TNB to the colorless DTNB again [20, 21].

$$SCN^- + H_2O_2 \xrightarrow{\text{peroxidase}} OSCN^- + H_2O.$$

The OSCN- ion is relatively stable and can be quantified by the oxidation of two sulfhydryl compounds, 5-thio-2-nitrobenzoic acid (TNB), to one disulfide compound (DTNB) [9, 18].

$$OSCN^- + 2R\text{-}SH \longrightarrow SCN^- + R\text{-}S\text{-}S\text{-}R + H_2O.$$

Each mol of DTNB upon complete reduction (cleavage of the disulfide bond) will yield 2 mols of TNB [22], and each 2 mol of TNB are reoxidized to the colorless DTNB again by 1 mol of hypothiocyanite [23].

Accordingly, each 2 mol of TNB (colored) is oxidized by 1 mol of hypothiocyanite (producing the colorless DTNB), so by knowing the concentration of TNB, we can measure the concentration of hypothiocyanite OSCN- [22].

Preparation of DTNB solution: One gram of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB Ellman's Reagent Molecular Formula ($C_{14}H_8N_2O_8S_2$), Molecular Weight 396.35, CAS Number 69-78-3, from Science Lab. Chemicals and Laboratories USA) was dissolved in 1000 ml of 0.1%

trisodium citrate (18) to obtain DTNB concentration of 2.5 mM.

preparation of 2-mercaptoethanol: one ml of 2-mercatoethanol (Fluka Chemicals, , formula HSCH2CH2OH, MW 78.13, net weight 1 kg, volume 1 liter) was mixed with 5 liters unionized DW in order to obtain 2.5 mM. [19]

Then 250 ml of each preparation were mixed and left for 3 hours at room temperature, shaked six times, once every 30 minutes [24].

After 3 hours, the color of mixture became yellow because of formation of 5-thio-2 nitrobenzoic acid (TNB) as a result of reduction of DTNB by means of 2-ME.

Measurement of TNB concentration:

The concentration of TNB was calculated by using computerized UV (VIS) spectroscope 1650 (PC)s type SHIMADZU whose cell path length is 1 cm, and the absorption (optic density) of TNB had been read against blank (trisodium citrate) at 412 nm assuming a molar extinction coefficient of 13.6 liter [25, 26]. Values of both absorptions were recorded on computer screen, and the net absorption was calculated.

Absorption of TNB A = A1-A2 = 0.05848

Concentration of TNB was calculated according to Beers Law [27]:

$A = € b c$

€ = molar extinction coefficient, b= cell path length, c= concentration of TNB which was found 4.3 mM.

Dilution of TNB: In order to measure different concentrations of salivary hypothiocyanite, TNB was diluted to obtain 17 samples (50 ml each) of TNB concentrations (TNB1 is 380 µM, TNB2 = 360 µM and so on until the least concentration TNB17 = 60 µM).

Quantification of hypothiocyanite in saliva samples:

The assay of measuring salivary OSCN- is based on oxidation of 2 mol of TNB (yellow) to the colorless DTNB by 1 mol of OSCN- [25], 0.5 ml of each saliva sample was mixed with the yellow mixture (containing known concentration of TNB) in the cold (ice water bath) for 5 minutes, this is the ideal method for rapid and complete oxidation of TNB [19, 22], when the mixture color disappears and becomes colorless, this means that the concentration of OSCN- is half the concentration of TNB, if not, this means that the concentration of OSCN- is less than half the concentration of TNB and so it was not able to oxidize all the TNB molecules of the sample, so the saliva sample was mixed in the same way with another TNB sample which has lower concentration.

All results were recorded according to the groups.

RESULTS

A - Results of serum anti-HSV1 IgG

Of 88 examined students, 20 were seronegative, 40 were seropositive with recurrences and 28 seropositive with out recurrence and they were grouped into:

1- **Group A:** Seropositive with RHL (no= 40)
2- **Group B:** Seropositive without RHL (no= 28)
3- **Group C:** Seronegative (no=20)

Results showed rise in level of anti-HSV1 IgG level in sera of seropositive subjects with recurrent labial herpes (86.8 EU/ml) than that in seropositive subjects without recurrence (47.2 EU/ml). (table 1, figure 1).

B- Results of salivary S-IgA

1- Group A: Salivary S-IgA mean level was 39 µg /min.
2- Group B: Salivary S-IgA mean level was 43.6 µg /min
3- Group C: Salivary S-IgA mean level was 42.3 µg /min (table 1, figure 2)

Results of chemical assay

Results revealed that the lowest concentrations of salivary hypothiocyanite were in group A and the highest concentrations were in group B (table 1).

1- Group A (no= 40): The mean salivary hypothiocyanite concentration in subjects of this group is 30 µM.
2- Group B (no= 28): The mean of salivary hypothiocyanite concentration in subjects of this group is 100 µM.
3- Group C: The mean concentration was 100 µM, as shown in table 1 and figure 3.

As shown in table 1, the anti-HSV1 IgG is higher in sera of symptomatic seropositive subjects than that in asymptomatic seropositive subjects and this means that although the level of anti- HSV1 immunoglobulin is high in serum but it is unable to prevent reactivation of the virus, also it is shown that in spite the low level of serum immunoglobulin in group B, but the virus is kept inactivated and subjects are asymptomatic seropositive, in other word, the serum anti- HSV1 IgG dose not play a significant role in controlling reactivation of the virus.

On the other side, results showed that saliva of subjects in symptomatic seropositive group (A) has the lowest concentration of hypothiocyanite, while subjects of asymptomatic seropositive group (B) showed the highest concentration of hypothiocyanite in their saliva. Also, it had been shown that salivary S-IgA is almost similar in

serpositive asymptomatic and seronegative groups but that of seropositive symptomatic was significantly lower.

The above results clarified that serum anti-HSV1 IgG is not efficient in preventing or controlling reactivation of the virus, while the natural salivary defense factor hypothiocyanite OSCN- plays the major role in controlling and preventing reactivation of the virus, and the efficiency of hypothiocyanite is depending on its concentration, so in group A, the low level of hypothiocyanite permits reactivation of the virus, but when hypothiocyanite concentration is high (group B), reactivation will be augmented, and a precise look on results revealed that when this concentration is equal to or exceeds (90) µM, no ractivation of virus will be noted.

This fact may give a hint about a threshold value level of hypothiocyanite concentration of (90) µM below which, this natural defense factor is unable to suppress virus reactivation.

Table 1: Results of selected parameters in 3 groups

	Sero-negative	Sero-positive asymptomatic	Sero-positive symptomatic (cases)
Serum Anti-HSV IgG antibodies concentration (EU/ml)			
Range	---	(41 to 56.3)	(77.1 to 107.9)
Mean	---	47.2	86.8
SD	---	3.6	8.8
SE	---	0.68	1.39
No	---	28	40
P (t-test) < 0.001			
2. Salivary S-IgA concentration (µg/min)			
Range	(34.1 to 50.8)	(34.8 to 51.9)	(32 to 46.9)
Mean	42.3	43.6	39
SD	4.5	5.2	3.9
SE	1	0.98	0.61
No	20	28	40
P (ANOVA) for difference in mean S-IgA concentration between the 3 groups < 0.001			
P (ANOVA) for difference in mean S-IgA concentration between:			
Sero-negative x Sero-positive asymptomatic = 0.33[NS]			
Sero-negativex Sero-positive symptomatic(cases) =0.007			
Sero-positive asymptomatic x Sero-positive symptomatic (cases) <0.001			
3. OSCN concentration (µM)			
Range	(60 to 140)	(90 to 120)	(30 to 70)
Median	100	100	30
Inter-quartile range	(70 to 110)	(100 to 110)	(30 to 60)
No	20	28	40
P (Kruskal-Wallis) for difference in median OSCN concentration between the 3 groups < 0.001			
P (Mann-Whitney) for difference in median OSCN concentration between:			
Sero-negative x Sero-positive asymptomatic = 0.39[NS]			
Sero-negative x Sero-positive symptomatic (cases) <0.001 [S]			
Sero-positive asymptomatic x Sero-positive symptomatic (cases) <0.001			[S]

--- ‹ 20 EU/ml which means negative according to leaflet of the kit

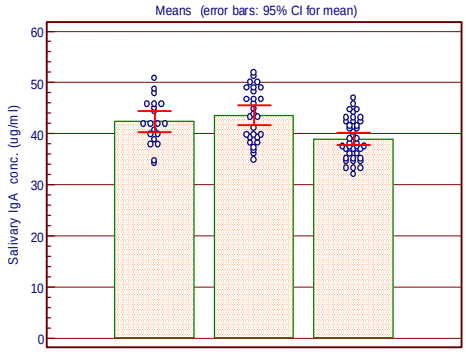


Figure 1: Serum IgG levels

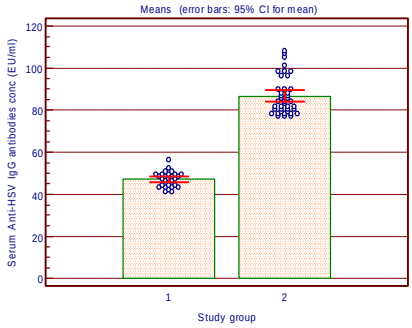


Figure 2: Levels of Salivary S-IgA

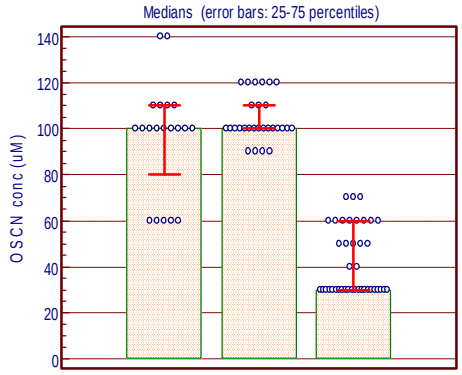


Figure 3: OSCN concentration (µM) in 3 study groups.

DISCUSSION

Serum anti-IgG level

1- Viral glycoproteins (gC, gE and gI) have characteristics of immune escape (28), an example of the immune escape function is gC which binds complement C3 protein and thus depletes it from the host's serum and inhibits complement-mediated reactions [29].

When the Fab domain of the antibody binds its target antigen, complement binds the Fc domain of the same antibody for enhancement of antibody-antigen neutralization, in HSV1 antigen,

2- viral glycoproteins E and I (gE & gI) bind the Fc domain of antibody and block it against complement so prevent neutralization of

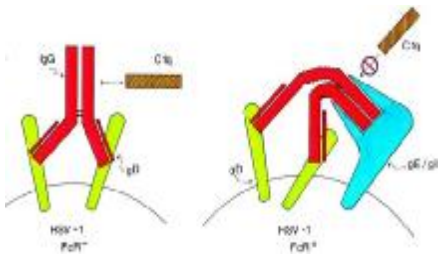


Figure 4: Viral glycoproteins bind FC domain of IgG preventing complement enhancement

the virus, thus, the virus remains covered with antibody but intact [30, 4].

3- Cell to cell viral spread:

The virus is able to spread from one cell to another without entering extracellular fluid, so it will not be exposed to antibodies [31].

These properties of the virus will render serum anti-HSV1 IgG ineffective against the virus whose reactivation will lead to increase in levels of this immunoglobuline.

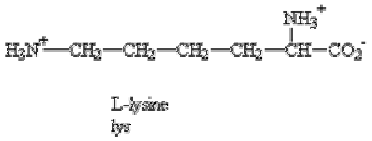
Salivary S-IgA:

Salivary S-IgA was higher in asymptomatic seropositive and seronegative groups than symptomatic seropositive. This may contribute to higher activation of peroxidase system because one of the biological functions of S-IgA is enhancement of peroxidase system [23], also SIgA was thought to prevent the penetration of virions into epithelial cells by sterically blocking cellular receptors [32].

Chemical assay

Results showed that the lowest concentration in group B (asymptomatic seropositive) was 90 µM which seems high enough to perform antiviral activity [11,34] by oxidizing the sulfhydryl group and proteins in glycoproteins of the viral envelope resulting in dysfunction of those glycoproteins, especially gB, gC, gD, gH and gI which are responsible for binding to the membrane of the host cell by herpes virus entry mediator receptor (HVEM) through gD and the cell surface particle heparan sulfate through gC [15].

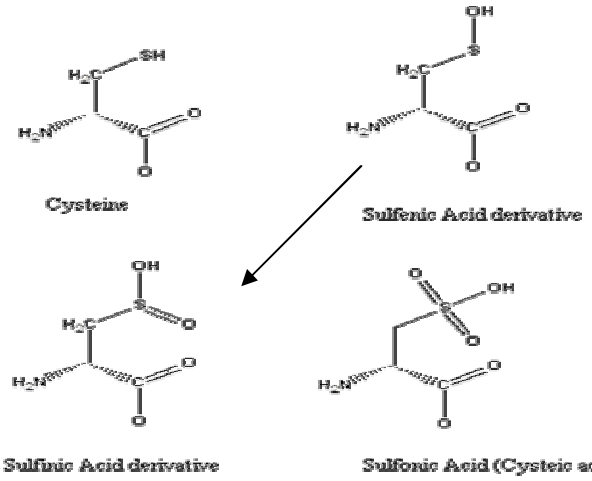
1-Heparan sulphate HS binding domain could be identified within gB based on analysis of the predicted amino acid sequence of the gB [33], studies of other HS binding molecules indicate that the negatively charged proteoglycan



molecules (HS) would be recognized by peptide domains rich in positively charged residues, particularly lysine [34, 35].

Hypothiocyanite oxidizes lysine residues leading to displacement of hydrogen from its side chain $NH3^+$ [34, 36] resulting in loss of the positively charged property of this domain of viral glycoprotein leading to impairment of its binding to the negatively charged HS receptor. The remaining amino group NH2 side chain will be exposed to further oxidation by OSCN- which has the characteristics to oxidize side chain amino group [37], maintained oxidation of amino group will result in irreversible destruction of the amino cid and subsequent change in amino acid sequence of the binding site polypeptide of viral glycoprotein [13, 38] resulting in inability of the binding domain of gB to recognize heparan sulfate receptor on the host cell membrane.

2- The most essential amino acid in gD is cysteine [39], OSCN- attacks this amino acid because it has amino side chain and oxidizes it. The first step of oxidation (sulfenic acid) is reversible by reducing agents. The next level of oxidation cannot be easily reversed and is almost final, once the protein has been altered in this manner the cysteine residue cannot be recovered [13].



The above oxidation reactions of hypothiocyanite affect most proteins in viral glycoproteins [40], and because gD and gC of the viral envelope represent the main apparatus of viral attachment to the cell wall of the host, and the importance of gD in penetration of the cell wall [15., 16, 31, 35], so dysfunction of those important glycoproteins means loss of path finder of the virus and render the virus incapable of binding to the host cell wall, and that is why those subjects have viral shedding from their saliva, but they still asymptomatic and did not experienced any recurrent labial herpes infection. In order to reach the irreversible step of viral protein oxidation, the oxidation should be maintained for more than one step, and this requires high concentration of oxidant (hypothiocyanite), and that is why the concentration of OSCN- should be ≥ 90 µM in order to act as antiviral factor, otherwise, the low concentration may perform the first step of oxidation, which is revesible, but cannot continue further oxidation steps, so the virus may recover again, reactivate, and cause recurrent infection, like what happened in seropositive group with RH

REFERENCES

1- Corey, Spear. Infections with herpes simplex viruses. N Engl J Med 2000; 314: 686–91; 749–57.

2- Miller, Danaher, Jacob. Molecular aspects of herpes simplex virus I latency, reactivation, and recurrence. Crit Rev Oral Biol Med 1998; 9:541–62.

3- Medveczky., Friedman & Bendinelli (Eds) Herpesviruses and Immunity; Plenum Press, 1998

4- Carr, Härle, Gebhardt. "The immune response to herpes simplex virus type 1 infection"; Exp. Biol. Med. (Maywood) 2001;226 (5): 353–66.

5- Spruance. Pathogenesis of herpes simplex labialis: excretion of virus in the oral cavity; J Clin Microbiol 2004; 19: 675–9.

6- Brock, Madigan, Martinko & Parker. (Eds.) Biology of Micro-organisms; Prentice Hall International, 8th Ed, 1996

7- Tateishi, Toh, Minagawa, Tashiro. Detection of herpes simplex virus (HSV) in the saliva from 1,000 oral surgery outpatients by the polymerase chain reaction (PCR) and virus isolation. J Oral Pathol Med 1994; 23: 80–4.

8- Mikola, Waris, Tenovuo. Inhibition of herpes simplex virus type 1, respiratory syncytial virus and echovirus type 11 by peroxidase-generated hypothiocyanite; Antiviral Res 2005; 26: 161–71.

9- Bates T, Jefferson. Hypothiocyanite Ion: Detection of the Antimicrobial Agent in Human Saliva; J Dent Res 2001;59:1466-72.

10- Morrison, Allen. Lactoperoxidase: Identification and Isolation from Harderian and Lacrimal Glands. Science 1996;152: 1626-8.

11- Morrison, Steele. Lactoperoxidase, the Peroxidase of the Salivary gland in Biology of the Mouth, P. Person, Ed., Washington, D.C.; American Association for the Advancement of Science, 1998, pp. 89-110.

12- Aune, Thomas, Morrison. Lactoperoxidase-catalyzed Incorporation of Thiocyanate Ion into a Protein Substrate. Biochemistry 1999; 16: 461-5.

13- Bjorck, Claesson. Correlation Between Concentration of Hypothiocyanite and Antimicrobial effect of the Lactoperoxidase System Against Escherichia coli. J Dairy Sci 1998; 63: 919-22.
14- Jacangelo, Olivieri. Interactions of hypothiocyanous acid and hypothiocyanite with pyrimidine nucleotides, and other substrates; Arch Biochem Biophys 1998;349:183-91.
15- Carfi, Willis, Whitbeck, Krummenacher, Cohen, Eisenberg, Wiley. Herpes simplex virus glycoprotein D bound to the human receptor HveA. Mol Cell 2001; 8: 169–79.
16- Cai Person, Warner Zhou, DeLuca Linkerinsertion. Nonsense and restriction-site deletion mutations of the gB glycoprotein gene of herpes simplex virus type 1. J Virol 1997; 61: 714–21.
17- Roizman, Sears. An inquiry into the mechanisms of herpes simplex virus latency. Ann Rev Microbiol 1997; 41: 543–57.
18- Prütz DTNB interactions and biologic uses. Arch Biochem Biophys 1996; 332: 110-20.
19- Rakita, Michel, Rosen. Redox technical levels. Chemistry 1989; 28: 3031-6.
20- Barrette, Hannum, Wheeler, Hurst. General considerations in chemical methodology. J Biochemistry 1989; 28:(9) 44-17.
21- Rosen, Rakita, Waltersdorph, Klebanoff. . Common and uncommon in vitro redox problems. J Biol Chem 1987;5:223-31.
22- Dychdala. Disulfide compounds properties and biological uses; 1991;p. 131-151. In S. S. Block (ed.). Lea & Febiger, Philadelphia.
23-Tenovuo Moldoveanu, Mestecky Pruitt, Mansson-Rahemtulla. Interaction of specific and innate factors of immunity: IgA enhances the antimicrobial effect of the lactoperoxidase system against Streptococcus mutans. J Immunol 1982;128:726–31.
24- Bacon, Irwin. Limiting factors in redox reactions. J Chem SOC 1998; 778-90.
25- Tenovuo, Anttonen. Peroxidase-catalyzed Hypothiocyanite Production in Human Salivary Sediment in Relation to Oral Health. Caries Res 1998;14:269-75.
26- Whittenburry. Hydrogen Peroxide, Accumulation of OSCN and Catalase Activity in the Lactic Acid Bacteria. J Gen Microbiol 2002; 35: 13-26.
27- Ingle, Crouch Spectrochemical Analysis; Prentice Hall, New Jersey (1988)
28- Kuby "Immunology" W.H.Freeman, 1997
29- Martin, Palmer, Kissling. Complement-fixing antigens of herpes simplex virus types 1 and 2: reactivity of capsid, envelope and soluble antigens. Infect Immun 2002; 5: 248–54.
30- Basu, Dubin, Basu, Nguyen, Friedman. Characterization of regions of herpes simplex virus type 1 glycoprotein E involved in binding the Fc domain of monomeric IgG and in forming a complex with glycoprotein I. J Immunol 1995;154:260–7.
31- Dingwell, Brunetti, Hendricks, Tang, Rainbow, Johnson. Herpes simplex virus glycoproteins E and I facilitate cell to cell spread in vivo and across junctions of cultured cells. J Virol 1994; 68: 834–45.
32- Burgio, Lanzavecchia, Plebani, Jayakar, Ugazio. Ontogeny of secretory immunity: levels of secretory IgA and natural antibodies in saliva. Pediatr Res 1980;14:1111–4.
33- Lingen Seck, Weise Falke. Single amino acid substitutions in the glycoprotein B carboxy terminus influence the fusion property of herpes simplex virus type 1. J Gen Virol 1995; Jul: 76: 1843–9.
34- Domigan, Vissers, Winterbourn. Modification of oral microbes by hypothiocyanous acid and hypothiocyanite. Redox Rep 1997; 3: 263-71.
35- Dolter Goins, Levine Glorioso. Effect of induced oxidation on type-specific antigenic determinants of herpes simplex virus glycoprotein C. J Virol 1992; 66: 4864–73.
36- Aune Thomas. Oxidation of Protein Sulfhydryls by Products of Peroxidase-catalyzed Oxidation of Thiocyanate Ion. Biochemistry 2002; 17: 1005-10.
37- Hazen d'Avignon, Anderson Hsu, Heinicke. Human neutrophils employ the myeloperoxidase-hydrogen peroxide-thiocyanite system to oxidize α-amino acids to a family of reactive aldehydes. J Biol Chem 1998; 251: 1145-56.
38- Hoogendoorn Piessense, Scholtes Stoddard. Hypothiocyanite Ion; The Inhibitor Formed by the System Lactoperoxidase-Thiocyanate-Hydrogen Peroxide. Caries Res 2000; 11: 77-84.
39- Watson Weis, Salstrom Enquist. Herpes simplex virus type-1 glycoprotein D gene: Pathogenicity of nucleotide sequence and expression. Science 1998; Oct: 22: 218(4570):381–4.
40- Scholtes Stoddard. Hypothiocyanite Ion; The Inhibitor Formed by the System Lactoperoxidase-Thiocyanate-Hydrogen Peroxide. Caries Res 2000; 11: 77-84.