

# Estimation of Salivary IgA in Hemophilic Patients under Treatment

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**Abstract:** Background: Hemophilia is a group of inherited coagulation disorders that considered as a serious congenital coagulation factor deficiency include : hemophilia A,B,C,the former one called the,classic one that result from factor VIII deficiency, second type result from factor IX deficiency called Christmas disease,the last one due to factor XI deficiency, the study establish relation between hemophilic patients and their immunological status through measurement of salivary IgA level while under treatment. Objective: The present study will be designated to determine the level of salivary IgA in hemophilia patients, make comparison with healthy control. Results: Level of salivary IgA was significantly decreased in hemophilic patients under treatment in comparison to healthy control. Conclusion: declination inthe level ofimmunological Marker (salivary IgA) in hemophilic patients as compared to normal healthy individuals.

**Keyword:** hemophilia,immunological marker(salivary IgA )

## 1. Introduction

There are two major antibody classes in saliva: SIgA and IgG and it continuously influence Oral microorganisms, SIgA is synthesized as pIgA by plasma cells in salivary glands and is exported by an epithelial receptor-mediated mechanism, along with the paracellular leakage of monomeric IgA and IgG antibodies. (Olayanju,*etal.*,2012).

The chief defense function of SIgA referred to as immune exclusion which appears simply to bind to soluble or particulate antigens, coating of bacteria present in saliva with IgA can be directly demonstrated by immunostaining, IgA considered to provide containment of the microbiota and counteract invasiveness although this apparently does not inhibit bacterial growth (Napimoga,*etal.*,2011).

The mucosal immune system is characterized by epithelial export of locally produced immunoglobulin which released to the lumen as secretory IgA antibodies; the magnitude of the salivary IgA response was much lower than the corresponding plasma IgA response. However, although the half-life of plasma IgA is around 1 week, locally produced IgA is continuously exported to saliva and flushed away(Aase,*etal.*,2016).

The secretory immune system is under complex regulation which distinctly affected the activity of the different cell types involved in the formation of SIgA. However, a number of immunological phenomena induced by mucosal antigen exposure are poorly defined in humans,there is evidence to suggest that gut associated lymph tissue and mucosal associated lymph tissue do not equally contribute to the induction of secretory immunity in different regions of the body, so it is unknown whether enteric immunization is the best way to stimulate salivary IgA responses, In fact, the various salivary glands may have different preferences (Brandtzaeg,2007).

The levels of total IgA and specific antibodies in salivary fluids influenced by several variables, these include: protein loss during sample handling, the flow rate,, difficulties with reproducibility and standardization of immunoassays, and uncontrolled admixture of serum-derived monomeric IgA and IgG to the samples. Nevertheless, salivary secretions still have interesting scientific and clinical potentials.(Per, 2013).

Several oral bacteria produce enzymes that can selectively cleave SIgA1 in its extended (13-amino acid) hinge region, especially certain strains of *S.sanguinis* and *S. mitis*, also Porphyromonas Prevotella and Capnocytophaga species which involved in periodontal disease at least 60% of salivary IgA consists of the IgA1 isotype, and parotid antibodies to *S. mutans* occur predominantly in this subclass, whereas reactivity to lipoteichoic acid from *S. pyogenes* and to lipopolysaccharides from *Porphyromonasgingivalis* *B. fragilis* and *E. coli* is carried mainly by in the IgA2 isotype.(Parisotto,*etal.*,2011)

## 2. Subject Material and Methods

The study was performed in, children welfare teaching hospital, hemophilia department, medical city, in Baghdad, sample consist of:

Group 1: 30 hemophilic patients

Group 2 :30control,aged matched with group 1, all were male gender.

### 2.1 Test procedure

The salivary IgA will be investigated as the procedure prescribed the precoatedplate was Washed (micro titer plate)5 x with 250micro letter ELISA wash buffer and Carried out the test in duplicate.

1) 100 µl STD(standards) was add, to patient saliva samples, then incubated of the sample for1 hour, then shaking were done on a horizontal mixer, at room

- temperature, after that Aspirated and washed the wells 5 $\chi$  with 250  $\mu$ L ELISA wash buffer was done.
- 2) 100  $\mu$ L CONJ(conjugate; POD antibody) was added, also Incubated for 1 hour, shaken on a horizontal mixer, at room temperature, then,then De cant the content of the plate was done and washed the wells 5  $\chi$  with 250  $\mu$ l wash buffer, and 100  $\mu$ l SUB(TMB substrate)was added, incubated for 5-15 minutes at room temperature.
  - 3) 50  $\mu$ l STOP (ELISA stop solution)Added and mixed shortly.
  - 4) Determined of absorption with an ELISA reader at 450nm against 620 nm as reference.(If no reference wavelength is available, reading will only be at 450nm).if the extinction of the highest standard exceed the measurement range of the photometer,absorption must be measured immediately at 450 nm against 620 nm as reference .

For the calculation of the saliva values, the result from the micro plate reader must be multiplied by 2.000.

### 3. Result

#### IgA level in the studiedgroups:

Table(1) shows summary statistics for studied of IgA level parameter in two different groups (Controlled, and Hemophilic) such that, "Mean values, Standard deviation, Standard error of mean values, 95% confidence interval of mean values, and two extremes values minimum, and maximum of the original readings".

Respect to subject of [IgA level parameter], results shows that majority of mean value are accounted in controlled group, and according to estimation by interval for the population mean value, it could be conclude that studied parameter in control group are extremely different compared with Hemophilic group, since their upper bounded are recorded too far from lower bounded of the control group.

Figure(1)represents graphically plotting chart of mean values for studied readings regarding studied [IgA level] in different (controlled, and Hemophilic) groups.

With respect of testing statistical hypothesis, which says that readings on the subjects of studied parameter of Immunological IgA level are thrown from the same population (same responding), and that should be proved according to testing equality of variances and equality of mean values by applying "Levene and t-tests" respectively, and as illustrated in table (2).

A highly significant different at  $P < 0.01$  had been registered in testing equality of means for studied parameter, while no significant difference for testing of equality of variances in light of testing equality of variances.

For summarizes of preceding results, it could be concludes that Immunological IgA level are accounted strong indicators for characterized differences between (controlled, and Hemophilic) groups

#### Suggested Technique

For registered a meaningful a cutoff point for distinguishing among Hemophilic and controlled group's readings in light of studied parameter "IgA level", figure (3) illustrated graphically the unique iteration using Stem-Leaf plot concerning IgA level.

Since no extremes and outliers of studied readings are accounted either in Hemophilic or controlled groups, then a cutoff point should be the lower bounded of the controlled group, which are shared with some readings of Hemophilic group. The following table (3) illustrated a cutoff point that are based on the applying of the suggested technique concerning IgA level

### 4. Discussion

Secretory immunoglobulin A (sIgA) is the most predominant antibody found in saliva with biologic activity, which considered as a major source of antigenic material in the mouth. (Sookto, *et al.* 2013), ( Fawad,*etal.*,2017).IgA have synergistically action with other factors like lysozyme and lactoferrinat prevent adherence of any microbes, on enzymes neutralizion, toxins and viruses; (Jafarzadeh *et al.*, 2010).

In salivary fluids the measurment of total amount IgA and other specific antibodies are affected by several variables, which include: during sample handling there will be a protein loss,the effect of flow rate,, difficulties with reproducibility and standardization of immunoassays, and uncontrolled admixture of serum-derived IgA and IgG to the samples.(Per Brandtzaeg, 2013)

Importantly, the numbers of antibodies transported through premised routes might be affected by certain oral conditions, for example gingival and mucosal inflammations, also by the integrity of the mucosal, acinar epithelial barrier (Brandtzaeg,2007)

Another important variables are geography and age,various stressors reportedly influence the IgA levels in different manners.( Booth,2009)

Another study cosirning IgA was done by Faris in 2015 they found out that Salivary immunoglobulin reflects the functional capacity of the glands,increased concentration of this component is usually marker of a poor general condition.

Aida,etal done arecentstudyin 2017 conserning IgA and other salivary biomarkers like Cytokines like interlukines and TNF- $\alpha$  & IFN- $\gamma$ and found that these seem to be the most used biomarkers involved in diagnostic and prognostic of oral diseases like Periodontitis and caries risk through immunoassays .

Difference between the level of IgA in hemophilic patients and normal control could explained by the fact the change in oral microflora in hemophilic patients also the level of IgA could represent the functional capacity of the salivary gland and its salivary flow rate which reflect poor general health.

**Table 1:** Statistics of IgA level in studied groups (Controlled & Hemophilic)

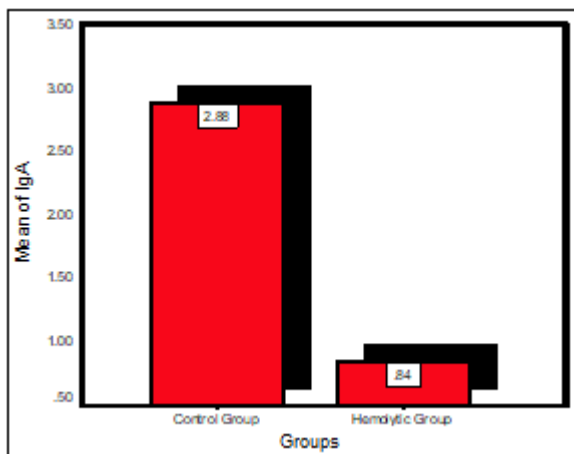
Parameter	Testing Homogeneity of Variances		t- test for equality of means			Mean Diff.
	Levene Statistic	Sig. (*)	t-test	d.f.	Sig. (*)	
IgA	1.163	0.285 (NS)	12.964	58	0.000 (HS)	2.043

**Table 2:** Comparisons Significant between Controlled and Hemophilic groups concerning Immunological IgA level

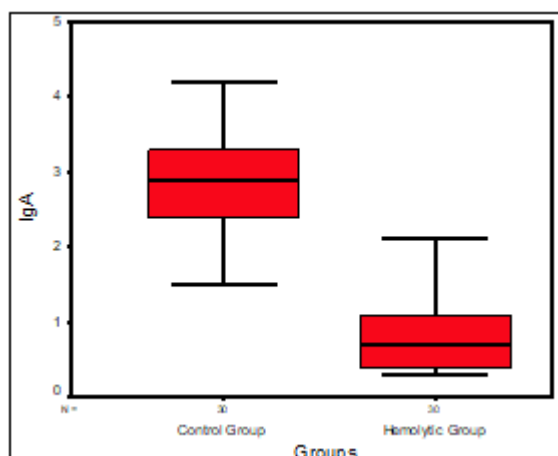
IgA	No.	Mean	SD	SE	95% C.I. for Mean		Min.	Max.
					Lower Bound	Upper Bound		
Control Group	30	2.8833	0.670	0.122	2.633	3.134	1.5	4.2
Hemolytic Group	30	0.8400	0.544	0.099	0.637	1.043	0.3	2.1

**Table 3:** Estimation cutoff point concerning Immunological IgA level

Studied Parameter	Cutoff point
IgA	1.50 (Min. of Controlled readings)



**Figure 1:** Bar Chart of mean values of Immunological IgA level in (Controlled & Hemophilic) groups



**Figure 2:** Stem-Leaf plot for estimating cutoff point concerning Immunological IgA level

## 5. Conclusion

Immunological state of hemophilic patients show declination through the low level of immunological marker (salivary IgA) compared to normal control group.

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