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# Glycophorin-A Mutations as a Window to Study Carcinogenesis

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**ABSTRACT** For risk assessment, it is necessary to evaluate the dose of human exposure to mutagens. Previous studies support the somatic mutation theory of carcinogenesis. It is therefore of great importance to have a simple mutation assay technique that enables screening of a large number of samples at low cost without much need for equipment inputs. Mutations at the human Glycophorin-A locus have been well documented in exposed populations and apart from providing lifetime dosimetry, it can also throw light on the condition of tumor suppressor genes whose integrity is of great value in the nature of carcinogenesis. Recently, it was thought that mutational mechanisms as that occurring at the human Glycophorin-A locus also can give rise to changes in tumor suppressor genes that are associated with oncogenes. They will reflect the probability of indication of tumor suppressor gene mutations. A study was conducted on control and samples from persons affected with an advanced stage of prostrate cancer. Glycophorin-A mutations were detected using the RS-1 assay. DNA from the same samples were prepared and were PCR amplified with the p53 gene at exons 6 and 7 and mutations looked at. Our results from the PCR amplified products matched perfectly with that of the results as obtained by the RS-1 assay. This proves that there is a correlation between between the Glycophorin-A mutations and mutations at the p53 gene and further makes the RS-1 assay a versatile technique for human health risk assements.

## INTRODUCTION

Mutations at the somatic cell level have been of great importance for Biodosimetric analysis of high-risk individuals. Detection and evaluation of somatic cell mutations are important, as these mechanisms can be possible predictors of cancer risk (Akiyama 1995). There are currently new and advancing opportunities for biological dosimetry of high-risk groups in methods of measuring somatic mutations (Mendelsohn 1990). Glycophorin-A gene mutations are one such method for biodosimetry and health risk assessment. The Glycophorin-A protein of human erythrocyte is a sialogylcoprotein with a chain of 151 aminoacids and occurs in two forms, the M and N. This is inherited in a Mendalian mode and the possible phenotypes are homozygous individuals with NN or MM and heterozygous MN. The M and N forms vary in their aminoacid composition in positions (1) and (5) (Furthmeyer 1978). The heterozygous individuals who constitute about 50% of the population are the subjects of study for the Glycophorin-A mutation studies. Increased mutation frequencies were detected hitherto at this locus among high-risk groups and cancer patients (Akiyama 1991). The mutant cell detected in this assay arise as a result of loss of function mutations and such mutational mechanisms also give rise to changes in tumor suppressor genes that are associated with oncogenesis. Thus, it was recently reported that mutant cells at the Glycophorin-A locus would reflect the probability of the induction of tumor suppressor gene mutations (Twan 2003). This theory gains support from the analysis of mutant cell frequencies in A-bomb survivors, where a significantly higher dose response was found for persons diagnosed with cancer than for cancer-free individuals (Kyoizumi 1996).

Techniques for detection of mutations at the Glycophorin-A locus were developed (Langlosis 1986) with the production of two monoclonal antibodies against the two variant forms of the human Glycophorin-A protein (Bigbee 1983). The techniques went through a series of changes and evolved into efficient methods of detection and quantification of the mutant cells. Recently, we

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reported a rapid and sensitive assay for the detection of variant erythrocytes, the RS-1 assay (Ravi 2002). RS-1 assay was employed in this study to detect the mutations at the Glycophorin-A locus.

P53 gene acts as a tumor suppressor gene and somatic mutations at the p53 locus have been implicated as casual events in the formation of a large and ever increasing number of common tumors including those involving the hematopoietic organs, and other common tumors. Infact, p53 is documented as the most frequently mutated gene in human cancers (Weinberg 1991).

We therefore thought it pertinent to find an association for the Glycophorin-A mutations with those in p53 locus as both have proved to be of value as predictors of cancer risk and oncogenesis. We analysed five control samples as controls from MN heterozygous individuals with no known exposure to mutagens and recorded the findings. Ten samples from persons of heterozygous MN with an advanced stage of prostrate cancer were analysed for the Glycophorin-A mutations. RS-1 assay was used for the above samples. The control samples showed a minimal shift in the RS-1 patterns and the cancer samples showed higher shifts, thus indicating a significant mutation occurrence. The samples were also subjected to DNA amplification studies. DNA was isolated from peripheral lymphocytes of the same samples and was *invitro* amplified by Polymerase chain reaction at the p53 locus at exons 6 and 7. Mutations at the p53 locus at exons 6 and 7 were observed using electrophoresis and Ethidium bromide staining. In the control samples, both exons were amplified but in the cancer samples, they were not amplified indicating the mutations at that locus.

This study throws light as to the association between the mutations at the human Glycophorin-A locus and the p53 locus. This further makes the RS-1 assay a versatile technique for human health risk assessments by virtue of it being a simple, rapid and low-cost technique.

## MATERIALS AND METHODS

*Glycophorin A mutational Assay:* One milliliter of venous blood was collected heparinised with prior informed consent from previously determined MN heterozygous individuals with no known exposure to mutagens and were considered as control samples. Such samples were also collected from ten persons with an advanced stage of prostrate cancer. The mutations at the Glycophorin-A locus were observed through the RS-1 assay as previously described (M.Ravi, 2002). Briefly, blood samples were washed thrice with cold saline and a final 10% erythrocyte suspension was prepared in saline. 50 micro liters of this suspension was used uniformly for the assay and were subjected to a series of antibody dilutions. The plates were observed after one hour for the pattern of Heamagglutination and button formations. The assay was performed in Heamagglutination plates and the results were interpreted as a factor of the antibody dilutions where agglutination ceases and button formation starts.

*P53 Gene Mutation Detection:* High molecular weight DNA was prepared from the peripheral blood samples using high salting out method and purified further by PCI (Phenol: Chloroform:Isomethyl alcohol) extraction method. The quality and quantity was checked by Agarose Gel Electrophoresis and spectrophotometrically. The ratio of 1.8 (A260 / A 280) quality DNA samples was subjected further to PCR DNA amplification of p53 gene at exons 6 and 7. The amplicons were subjected to Agarose Gel Electrophoresis (2% Agarose) and analysed further to staining with Ethidium bromide. The band patterns were analysed by comparing with a known molecular weight 100 base pair DNA ladder.

## RESULTS

The results as obtained in the RS-1 assay for the control samples show a minor shift in the agglutination patterns, thus indicating base line mutations. The results are given in Table 1. A significant shift in the patterns was observed in the cancer samples as given in Table 2. This indicates a considerable level of mutations at the Glycophorin-A locus in cancer samples. A representative picture of the RS-1 patterns for the samples is given in Figure 1. Intact amplified bands at the exons 6 and 7 of the p53 locus were observed for the control samples in the amplicons of control samples as observed from Agarose Gel Electrophoresis stained with Ethidium Bromide. The bands were absent in similar observations of cancer samples, an indication of mutations at the p53 locus at exons 6 and 7 as indicated in Figure1.

**Table 1: The results obtained as Heamagglu-tination patterns in the RS-1 assay performed on control samples indicating minor base-line mutations.**

| *Antiserum* | | *40* | *30* | *20* | *10* | *9* | *8* | *7* | *6* |
|---|---|---|---|---|---|---|---|---|---|
| *Saline* | | *60* | *70* | *80* | *90* | *91* | *92* | *93* | *94* |
| A | M | + | + | + | + | + | + | - | - |
| | N | + | + | + | + | + | - | - | - |
| B | M | + | + | + | + | - | - | - | - |
| | N | + | + | + | - | - | - | - | - |
| C | M | + | + | + | + | - | - | - | - |
| | N | + | + | + | - | - | - | - | - |
| D | M | + | + | + | + | + | - | - | - |
| | N | + | + | + | - | - | - | - | - |
| E | M | + | + | + | + | + | - | - | - |
| | N | + | + | + | + | - | - | - | - |

+ Agglutination
- Button formation

**Table 2: The results obtained as Heamagglutination patterns in the RS-1 assay performed on erythrocyte samples from prostrate cancer patients indicating significant mutations at the Glycophorin-A locus**

| *Antiserum* | | *50* | *40* | *30* | *20* | *10* | *9* | *8* | *7* | *6* | *Remarks* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Saline* | | *50* | *60* | *70* | *80* | *90* | *91* | *92* | *93* | *94* | |
| PCa 10 | M | + | + | + | + | + | + | + | - | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 11 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 12 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 13 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 14 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 15 | M | + | + | + | + | + | + | + | _ | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 16 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 17 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 18 | M | + | + | + | + | + | + | + | + | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |
| PCa 19 | M | + | + | + | + | + | + | + | - | - | Significant |
| | N | + | - | - | - | - | - | - | - | - | shift |

+ Agglutination
- Button formation

## DISCUSSION

Human erythrocytes, devoid of biosynthetic and repair capabilities are a good cellular source to study mutations as they provide us with unaltered and *prima face* evidence of mutations at the Glycophorin-A locus. These protein molecules are present as $5 \times 10^5$ copies per cell of normal erythrocytes and the genes regulating their expression are susceptible to mutations, which reflect as a loss-of-function mechanism. Changes in the peripheral circulating erythrocytes reflect the mutational events at the long-lived hematopoietic stem cells and thus give us a life-long, cumulative mutational record. As the Glycophorin-A molecules are expressed on the surface of the erythrocytes, and by virtue of their antigenic nature, are perfect targets for which it is possible to produce suitable antibodies in a phylogenetically distant host. Monospecific, agglutinating antibodies for the two forms of the proteins are available and monoclonal antibodies for the same were also previously reported . As these protein molecules are transmembranic, it is possible to agglutinate the intact erythrocytes with the use of specific antibodies. These antigens are co dominantly expressed on the erythrocyte surface and can be exploited by

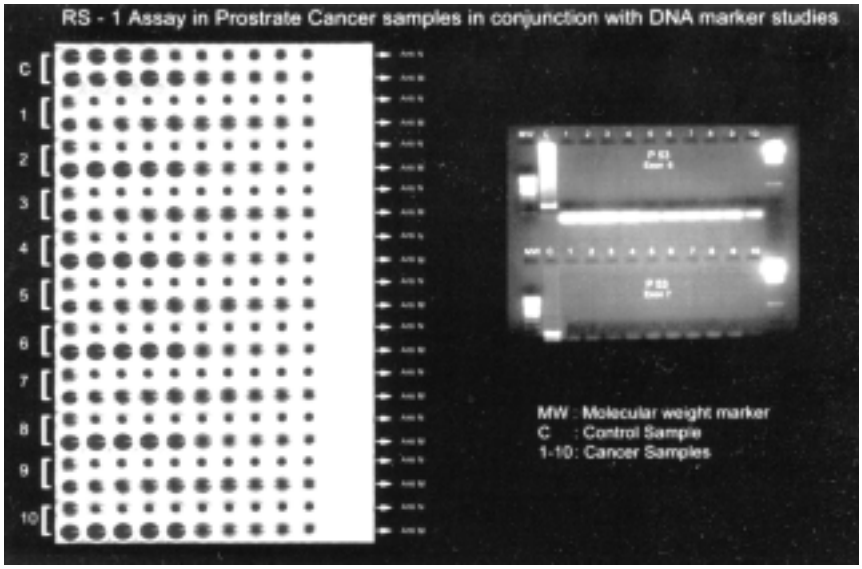


**Fig. 1. RS-1 Assay and DNA amplification studies were carried out for samples collected from patients with an advanced stage of prostrate cancer. The RS-1 patterns and the amplicons showed similar results indicating a correlation in mutations between the Glycophorin A and the P53 loci.**

simple Hemagglutination as in RS-1 assay to provide us with an indication of the loss of a particular type of protein and thus mutations at the Glycophorin-A locus in heterozygous individuals.

## CONCLUSION

Our simultaneous studies of mutations at the human Glycophorin-A locus and at the p53 locus at exons 6 and 7 indicate a good correlation between the two suggesting the versatility of the RS-1 assay as a simple tool for human health risk assessment.

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