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RESEARCH ARTICLE

ISOLATION, QUALITATIVE AND QUANTITATIVE ESTIMATION OF RNA FROM DIABETIC AND PREDIABETIC SUBJECTS

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ABSTRACT

RNA was isolated from Blood samples of various subjects of Diabetes, prediabetes and healthy individual. The entire DNA/RNA that has been extracted was found to differ in their molecular weight and was seen as separate bands when viewed under UV light. The concentration of the RNA was estimated by using the Nanodrop 8000 spectrophotometer. The concentration of healthy subject RNA was found to be more from Diabetic and prediabetic the ratio of their absorbance at 260 and 280 nm, was 2.01 which showed purity in the sample. The concentration of RNA was found to be the least in Prediabetic subject and ratio was found to be 1.89 indicating the presence of contamination. The RNA isolated from the Diabetic having ratio 1.97 which also indicates slight contamination.

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INTRODUCTION

We are fast entering a golden era of comparative genome analysis (Watson et al., 1953). The systematic comparison of different animal genomics gives a chance of identifying genetic basis for diversity. There are evidences says DNA determines the amino acid, but does it directly participate in protein synthesis? There are variety of proofs indicates that while RNA seemed to be directly involved, DNA is not directly involved in Protein Synthesis. It was shown in 1940 by Brachet and Caspersson that the amount ofprotein synthesis was directly correlated with the amount of cellular RNA, and that RNA was present both in cytoplasm and nucleus. The involvement of RNA in protein synthesis was more directly demonstrated by treating the cells with ribonucleases which degrade the RNA present in the cells. Ribonucleic Acid is an almost universal macromolecule used to study their transcriptional factor. The methods of molecular biology depend upon an understanding of the properties of biological macromolecules. The living cell is an extraordinarily complicated entity producing thousands of different macromolecules and harboring a genome. Principle behind the separation of macromolecule which is present in the cells is to make free from the other cellular components (Saenger, 1984).

Methods used to isolate the macromolecule (RNA) depend on the source, age and size of the sample. Isolation of RNA is needed for the genetic analysis, which is used for scientific, medical or forensic purpose. Scientists use RNA in a number of applications, such as introduction of RNA into the coding of protein synthesis in cells, animals or plants, or for diagnostic purposes. (Ammayappan et al., 2011). Many protocol have been used for isolation of RNA, but because of chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some case fail to respond to the same protocol. The present study deal with the modern approaches to develop a simple study of RNA isolation, their quantification and qualitative estimation of RNA extracted from different subjects for the molecular biological study of Diabetic and prediabetic subjects and to correlate with metabolic aspects in the said disease.

MATERIAL AND METHODS

Reagent and chemical: Phenol in saturated buffer 380ml, Guanidine thiocyanate 94.53 g, Ammonium thiocyanate 76.12 g, Sodium acetate, pH 5.0 33.4 ml of 3M stock, Glycerol 50 ml, DEPC to adjust final volume to 1L (above mentioned

chemicals are used in Trizol Method for RNA isolation). 0.8M sodium citrate/1.2 M NaCl, Isopropanol, Chloroform, 75% ethanol prepared with DEPC –Water, RNase Inhibitor. 1% Formaldehyde Denaturing Agarose Gel (for Quality Check), Nanodrop 8000 Spectrophotometer (for Quantity check).

RNA Extraction from Blood Sample

Qualitative Estimation of RNA: 1% formaldehyde denaturing agarose gel electrophoresis was used to separate RNA fragments. The sample was mixed with ethidium bromide, a marker dye and then loaded well in agarose gel which was then kept in position in the electrophoresis chamber filled with buffer and current was applied. (figure1). When the marker dye approached the end of the gel, the current was stopped and viewed under ultra violet light (Moyo et al 2008).

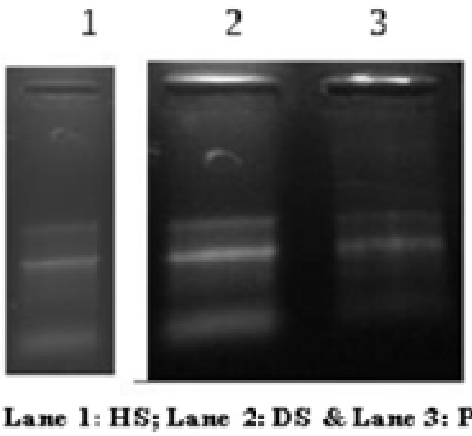
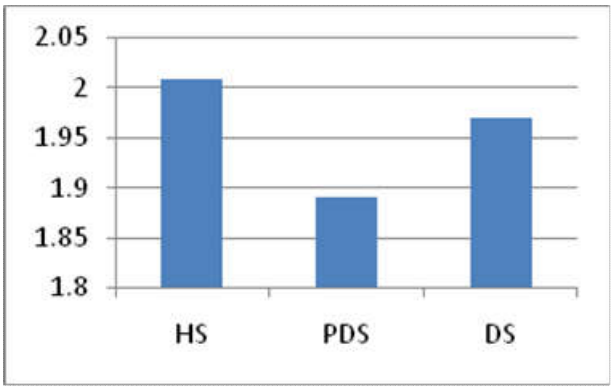


Figure 1. 1% Formaldehyde denaturing Agarose Gel Electrophoresis Lane1: HS (Healthy Subjects), Lane 2: DS (Diabetic Subjects, Lane3: PDS (Prediabetic Subjects).

Quantitative Estimation of the purity of the RNA: Dissolve Ribonucliec acid in 5mM NaOH solution to prepare standard solution. Mix equal volume of stock RNA solution with 1 N perchloric acid and heat it for 15min. at 70^0C. Measure absorbance at 260nm and 280nm using a Nanodrop 8000 spectrophotometer of different concentration of standard RNA solution plot a graph or standard curve. Usually, the absorbance is measured at 260 nm at which wavelength of absorbance A 260 of 1.0 corresponds to 50 µg take a small volume of RNA extract in test tube and put into 0.5 N perchloric acid so that it may be diluted and contain 0.02-0.25m mole of DNA-phosphate/ml. take 2 ml of RNA sample in a test tube and add 4 ml of DPA reagent. Incubate the mixture at 25-30^0C for 15-17 hrs. Measure absorbance of the solution at 260 and 280nm after calibrating the spectrophotometer with blank tube and standard containing the similar volume of perchloric acid.(Dubey et al 2008) As the RNA concentration increased the OD value also increased. OD is directly proportional to RNA concentration (Psifidi et al 2010).

RESULTS AND DISCUSSION

The differences quality and quantity of isolated RNA observed in gel electrophoresis and stained with ethidium bromide. The dye intercalates into RNA, and the intensity of fluorescence induced by UV light is proportional to the amount of RNA in the corresponding lane. (Figure 1). Of HS (Healthy Subject), DS (Diabetic Subjects) and PDS (Prediabetic Subjects). The concentration of the RNA was estimated by UV spectrophotometer using the formula 50 X OD X´ 20 µl/4000 and the values were recorded in Table 1. The concentration (A260/280) of RNA was found to be more from HS (Healthy Subject) and the ratio of their absorbance at 260 and 280 nm is 2.01 which showed purity, the concentration of RNA was found to be least from PDS (Prediabetic Subject) and ratio was found to be 1.89 showed the presence of impurity (Table 1). The RNA which was isolated from the DS (Diabetic Subject) was found to be slightly pure which was free from certain undetermined factor the concentration obtained was 1.97. (Ammayappan et al.,2009). purity were compared by standard chart (Table 2).



Graph 1. A260/280 1= HS, 2= PDS, 3= DS

Table 1.

Sample ID	Concentration (ng/µl)	Yield (µg)	A260/280
HS	278	5.5	2.01
PDS	242	4.8	1.89
DS	326	6.5	1.97

Table 2. Standard Ratio used for macromolecules

Purity of	Target A260/280 Ratio
DNA	1.8
RNA	2.0
Protein	0.6

In molecular biology, isolation of purified RNA is required for numerous manipulations. For large scale RNA isolation, several procedures relatively rapid mini preparations have been developed. The time required for extraction and purification depends upon the purity of RNA and its suitability for various procedures such as restricted enzyme cleavage, ligation and cloning (Mandelkern et al., 1981) extracted and purified RNA is going to utilized for further process in WTA (whole Transcriptome analysis), to examine the differential gene expression of said subjects.

Conclusion

Technical revolution in the field of genetics has allowed identification of numerous genetic variants that associate with T2D. The genetic landscape of T2D susceptibility is as yet incomplete, thus far only explaining a small proportion of the total heritability of diabetes. Many possibilities to dissect the architecture of T2D etiology have emerged in the form of large-scale genetic studies, meta analyses and sequencing in

families. If has already greatly contributed to our understanding of disease mechanisms by identifying pathways that could not be linked to diabetes by existing hypothetical models, even though many genetic findings are very recent and have yet to make their contribution to our knowledge about diabetes pathogenesis. However, one must bear in mind that diabetes is probably a much more diverse disease than the current subdivision into T2D implies and more precise subdivision into subgroups may both facilitate the investigation of T2D genetics and pave the way of more indivdualized treatment.

In conclusion these results show that purity of RNA is based on A260/280, HS , PDS and DS.. The isolated RNA was separated by gel electrophoresis and measures the total estimation of nucleic acid. This method involve measuring absorbance at 260 and 280 nm .A good quality RNA sample should have a A260/A280 ratio of 1.9 – 2.0 is desired when purifying nucleic acid. A ratio less than 1.8 means there is probably contaminant in the solution, typically either protein or phenol. The A260/A280 ratio is a particularly robust method for assessing RNA/DNA contamination of protein preparations. Because RNA and DNA are so similar, spectrophotometry cannot be used to detect contamination of DNA by RNA. But with the above said procedure separation of macromolecules can be done and purity can be specified. Purified RNA processed to WTA (Whole Transcriptome Analysis) to get the correlation of different subjects from the basis of pathway analysis and there chromosomal locations, also the Differential gene expressed in different subjects there working on upstream and downstream process.

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