



The Effect of Storage Temperature In Cooler Bag on Breast Milk Quality

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Abstract. Breast milk is a quality baby food because it contains nutrients and immunity. The high percentage of working mothers who breastfeed (92.5%) is directly proportional to the number of babies who consume pumped breast milk. It is feared that the storage of breast milk at a specified temperature and time can reduce its quality, including immunoglobulin A (IgA) levels. Objective: The objective of this study was to prove the effect of storage temperature on the quality of breast milk (IgA). Methods: This study used a quasi-experimental design with a pre-post-test design with a control group design. The sampling technique used purposive sampling of 32 nursing mothers in the experimental group and 32 nursing mothers in the control group included in the inclusion criteria (divided into control and experimental groups). The bivariate analysis utilized paired until t-test followed by independent t-test. Results: There was a decrease in IgA levels in the cooler bag storage temperature (4-8 °C) for 6 hours with an average difference of -18.264 ng/ml (p-value 0.012). IgA levels also decreased at room temperature storage (26 °C) for 6 hours with an average difference of -373.209 ng/ml (p-value 0.000). Different tests in the two groups had an average difference in IgA levels of -354.945 ng/ml (p-value 0.000), which meant significant differences. Conclusion: Storage temperature at cooler bag temperature 4-8 °C is better than room temperature of 26 °C on breast milk quality (IgA level).

Keywords: Back massage; foot soak; hypertension.



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Introduction

Breast milk is a quality nutrient for infants because of its nutritional and immunity content (1). Breast milk has to be maintained so that quality can optimize the baby's growth, development, and health(2). The proportion of children aged 0-23 months whose mother works breastfed is 92.53%(3). The high percentage of working mothers who breastfeed is precisely proportional to the number of babies who consume milk. For mothers who have problems in direct breastfeeding, alternating with family or caregivers when caring for their babies, and the advancement of breast milk pump technology is also the cause of housewives giving milk to their babies(4).

Storage of breast milk at a specific time and temperature becomes an unavoidable activity. Previous studies on the effect of temperature and storage time focused on the nutritional content of breast milk. Another ingredient that is just as important is immunity that is useful in preventing disease. A study showed that lactoferrin levels are stable, and lysozyme levels increase in storage temperatures of -200C for five days (5).

A study also proved that pasteurization temperature improved IgA, IgM, IgG, lactoferrin, lysozyme, lymphocytes, and cytokines (6). The principal immunoglobulin is found in breast milk, namely IgA.(7) IgA levels have not been studied. Knowing the changes in IgA levels in breast milk stored at a specific time and temperature is imperative to assess the feasibility of breast milk consumed to meet the baby's immune needs. Hence, researchers are interested in researching with the title, "The Effect of Storage Temperature on Cooler Bag on Breast Milk Quality: Immunoglobulin A."

Objective

The objective of this study was to prove the effect of storage temperature on the quality of breast milk (IgA).

Method

This research type used a *quasi-experimental research design* with a *pre-test post-test design with a control group design*^(8, 9). Researchers arranged two groups, namely the intervention group that was tested for IgA ASI at temperatures *cooler bag* (4-8 °C) at 0 hours (pre-test) and after 6 hours (post-test). The control group carried out the IgA ASI test at room temperature (26 °C) at 0 hours (*pre-test*) and after 6 hours (*post-test*). IgA levels were tested at 08.00 and 14.00 WIB, and in controlling the temperature in the *cooler bag*, temperature measurements were carried out at 0 hours (4 °C), 3 hours (6 °C), and 6 hours (8 °C). At room temperature, an incubator is used so that the temperature remains constant (26 °C). Checking IgA levels uses the ELISA Kit Elabscience instrument, while the instrument for measuring temperature is a *Cooler bag* with waterproof material, an incubator (room temperature controller), and a thermometer. In addition, other supporting instruments include an electric breast pump, breast milk plastic with BPA-free material, a watch, and hand sanitizer.

The population in this study were all breastfeeding mothers with a maximum infant age of six months who had visited the Mlati II Public Health Center, Sleman, DIY. Determination of the minimum sample size using techniques *non-probability sampling* with *purposive sampling method* and based on inclusion and exclusion criteria as many as 64 respondents divided into two groups with 32 respondents each in the intervention group and 32 respondents in the control group.

In this study, researchers collected data using observation, identification, interview, and filling out observation sheets. The collected data were analyzed through the IBM SPSS version 24.0 program and continued with different tests, namely the parametric test (*Paired t-*

test and independent t-test)⁽¹⁰⁾. The processed data is used as a basis in discussing the problem statement, which is then presented in tabular form so that conclusions can be drawn

Results

The mean difference of storage temperature in the cooler bag for 6 hours on the IgA breast milk level

IgA levels before and after the intervention with the storage temperature in the cooler bag (4-8 °C) for 6 hours decreased mean difference was -18,264 with the p-value was 0.012 (<0.05). It was indicated that there were significant changes in IgA levels before and after intervention with storage temperature in a cooler bag (4-8 °C) for 6 hours. In controlling the temperature in the cooler bag, temperature measurements are made at 0 hours (4 °C), 3 hours (6 °C), and 6 hours (8 °C).

Table 1 The mean difference of storage temperature in the cooler bag for 6 hours on the IgA breast milk level

Variable	Categorical	Mean ± SD	P-Value
IgA Breast Milk	Pre-test (0 jam)	1415.775±226.971	0.012
	Posttest (6 jam)	1397.512±231.525	

**Paired t-test*

The mean difference of storage temperature in the room temperature for 6 hours on the IgA breast milk level

IgA levels before and after the intervention with storage temperature at room temperature (26 °C) for 6 hours decreased the average difference of -373.209 with p-value in the experimental group was 0.000 (<0.05), which means there was a significant change in IgA levels before and after the intervention with storage temperature at room temperature (26 °C) for 6 hours. In controlling room temperature, an incubator is utilized to keep the temperature constant (26 °C).

Table 3.2 The mean difference of storage temperature in the room temperature for 6 hours on the IgA breast milk level

Variable	Categorical	Mean ± SD	P-Value
IgA Breast Milk	Pre-test (0 jam)	1427.650±231.829	0.000
	Posttest (6 jam)	1054.442±207.014	

**Paired t-test*

The mean difference of storage temperature between the cooler bag and room temperature for 6 hours on the IgA breast milk level

IgA levels in the respondent breast milk sample at the temperature of the cooler bag temperature (4-8 °C) with room temperature (26 °C) for 6 hours had an average difference of -354,945 ng/ml with a p-value of 0.000 (<0.05) which means that there are significant differences in IgA levels in the control group and the intervention group.

Table 3.3 The mean difference of storage temperature between the cooler bag and room temperature for 6 hours on the IgA breast milk level.

Variable	Group		P-value
	Intervention	Control	
	Mean ± SD	Mean ± SD	
ΔIgA	-18.264±39.550	-373.209±275.189	0.000

**Independent t-test*

Discussion

Effect of Storage Temperature on Cooler Bag for 6 hours on IgA Breast Milk

The results of the study showed the storage temperature in the cooler bag (4-8 °C) before and after storage for 6 hours decreased the average difference in levels of -18,264 ng/ml with a p-value of 0.012 (<0.05). It means Ho was rejected, meaning that there are significant differences in levels of IgA ASI before and after storage for 6 hours at cooler bag temperature (4-8 °C). This result is corroborated by research conducted by Arifin, which states that the coolant temperature (2-8 °C) influences the decrease in the quality of breast milk protein, including IgA levels composed of protein components⁽¹¹⁾. The research proves that storage at refrigerator temperatures for 2, 4, and 6 hours showed a significant difference with a p-value of 0.000 (<0.05) with an average difference of -0.02gr%⁽¹¹⁾.

The decrease in IgA levels after storing milk for 6 hours at a cooler bag temperature (4-8 °C) follows the theory, which states that storage time and temperature impact the concentration of breast milk content. Reduction in the activity of immunological components of breast milk, including IgA, was due to bacterial proliferation. A type of immunologic protein also supports it in IgA that is prone to low-temperature degradation, coupled with a storage time of 6 hours. Therefore, decrease in IgA levels by an average difference of - 18,264 ng/ml⁽¹²⁾.

As long as breast milk bacteria are at a low temperature, such as a cooler bag (4-8 °C), the bacteria still try to metabolize for its survival. Metabolic processes use protein, fat, and carbohydrates so that breast milk can be invoked as a microbial growth medium. In digesting nutrients in breast milk, bacteria secrete enzymes protease, lipase, and beta-galactosidase, harming anti-microbial proteins that convert free amino acids into toxic amines. The IgA component is composed of proteins, so that the process of microbial metabolism results in a decrease in IgA levels.^(13,14) In principle, low storage temperatures can only slow down the speed of metabolic reactions, enzyme activities, various chemical reactions, and the growth of microorganisms that cause damage but cannot prevent the overall⁽¹⁵⁾.

The Effect of Storage Temperature of Room Temperature for 6 Hours on the Level of IgA Breast Milk

All breastfeeding samples of respondents with a storage temperature at room temperature (26 0C) for 6 hours decreased IgA levels with a mean difference of -373,209 ng/ml. The average difference has a p-value of 0.000 (<0.05) which means that Ho is rejected, meaning that there are significant differences in IgA levels before and after storage for 6 hours at room temperature (26 0C). Research conducted by Arifin revealed that room temperature (25-28 0C) affects the decrease in the quality of breast milk protein, including IgA levels composed of protein components.

Changes in protein properties are caused by heat, acids, bases, organic solvents, salt, heavy metal, and radioactive radiation. Room temperature (26 0C) makes proteins undergo denaturation. Protein denaturation is meant by a change or modification of the secondary, tertiary, and quaternary structure of protein molecules without breaking covalent bonds. The process of breaking hydrogen bonds, hydrophobic interactions, salt bonds, and opening of denatured protein molecules causes a decrease in protein content, which is a part of IgA⁽¹¹⁾.

Another study conducted by Ruhana proved that the storage temperature has a significant impact on the parameters of protein content, including the components of the protein constituent IgA with a p-value of 0.000 (<0.05). At 24 hours of storage, the protein content decreased to 98.04% at room temperature. Room temperature can stimulate enzyme activity, chemical reactions, and microbial growth(16).

The mechanism of a significant decrease in IgA levels after six hours of breast milk storage at room temperature (26 °C) also starts from accelerating microbial growth due to its favorable temperature. Next comes adhesion (adhesion) and colonization. IgA then secretes IgA1 and IgA2. Microorganisms produce protease enzymes and break the exclusive bonds of proline-threonine or short-chain proline so that IgA is damaged and becomes inactive (17).

Storage Temperature Difference between Cooler Bag and Room Temperature for 6 Hours

Build on the study results, IgA levels in the respondent breast milk sample at the cooler bag temperature (4-8 °C) with room temperature (26 °C) for 6 hours had an average difference of -354.945 ng/ml. The average difference has a p-value of 0,000 (<0.05), which means that H_0 is rejected. There are significant differences in IgA levels in the control and experimental groups. This result is supported by research from Ruhana, which treats the storage temperature of breast milk at room temperature, coolant temperature, and freezer temperature. Within 24 hours, there was a decrease in breast milk protein levels as much as 98.04% at room temperature, a decrease in breast milk protein levels as much as 66.14% at cooling temperatures, and a decrease in breast milk protein levels as much as 30.5% at freezer temperatures. Storage temperature has a significant impact on protein parameters in breast milk with a p-value of 0.000. This protein component is also a constituent of IgA. The study results follow the principle of low-temperature storage that can slow the growth of microorganisms that cause damage. Inhibit enzyme activity and various chemical reactions (16). Breast milk is an excellent medium for microbial growth, which can be suppressed by storage at temperatures cooler than room temperature in a cooler bag.

Another study conducted by Arifin revealed an effect of storage at room temperature (room temperature 25-28 °C) on decreasing protein content in breast milk. There is a storage effect on the refrigerator (2-8 °C) on the decrease in protein content of breast milk. However, the impact of storage temperature on the fridge compared to room temperature has a p-value <0.05 , which means that storage in the refrigerator is better at maintaining protein levels, including IgA levels (11). The process of lysis mechanism or IgA inactivity is the same in both the control and experimental groups. However, in the experimental group, the temperature in the cooler bag was more able to suppress the process. In the control group, the decrease in IgA levels was more significant.

IgA production in mothers can adjust the needs of their babies. A found an estimated IgA level needed as much as 1105 ng/ml so that the storage temperature in the cooler bag (4-8 °C) for 6 hours is suitable for consumption by infants and able to maintain the stability of IgA levels with an average of 1397,512 ng/ml and a decrease of -18,264 ng/ml(18).

Newborns can not produce IgA independently in their bodies, so it still depends on the milk given. After three months, IgA starts to be processed in the baby's body, and after six months, IgA and other immune systems have worked optimally.

Storage of breast milk at room temperature (26 °C) for 6 hours was not able to maintain IgA levels with an average of 1054.444 ng/ml and decreased by 373.209 ng/ml. The impact that can occur if the level of IgA consumed by the baby is less, then the baby is susceptible to numerous diseases early in life. The role of secretory IgA as an antibody in fighting microbes is lower than optimal. The lack of protective function results in more

efficiently attaching viral and bacterial antigens to the gastrointestinal tract and respiratory tract. The baby is under a more significant potential for infection (19-20).

Conclusions and Suggestions

Storage of breast milk in cooler bag temperature (4-8 °C) before and after storage for 6 hours was significantly decreased on levels of IgA breast milk with a mean difference of -18,264 ng/ml, p-value 0.012. Storage of breast milk at room temperature (26 °C) before and after storage for 6 hours has a significant effect on decreasing levels of IgA breast milk (an average difference of -373,209ng/ml, p-value 0,000). There is a substantial difference in the temperature of the cooler bag temperature (4-8 °C) and the room temperature (26 °C) on the decrease in the level of IgA breast milk (the average difference in the storage temperature of the cooler bag is -18,264 ng/ml, at a room temperature of -373,209 ng/ml, p-value 0,000). Researchers can then consider the variations in temperature and time of the quality of breast milk.

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